WHO GUIDELINES ON USE OF MEDICALLY IMPORTANT ANTIMICROBIALS IN FOOD-PRODUCING ANIMALS

Web Annex A. Evidence base

World Health Organization

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1. SYSTEMATIC REVIEWS

1.1 Use in food animals of critically important antimicrobial agents for human medicine (Bond University, Australia)

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Executive Summary

Background

The contribution of antimicrobial administration to food animals to antimicrobial resistance in humans forms a potential risk to human health. The WHO is embarking on guidelines to advise member states. To support this, it commissioned a systematic review of the scientific literature to address the potential benefits of limiting antimicrobials for this purpose.

Questions

Do interventions for limiting use of antimicrobials in food animals reduce antimicrobial resistance in 1) other animals; *and* 2) humans?

Methods

Timeline: This was a rapid systematic review, undertaken in 4½ months.
Search strategy: This was built and tested with a validation set of already known relevant studies.
Screening: teams of trained personnel screened by title/abstract, and then by full-text; consistency between screeners was tested.
Data extraction: experienced teams extracted data into pre-designed and tested forms.
Synthesis: two experts undertook a narrative synthesis of the data. Heterogeneity (principally from different animals, settings, antimicrobial classes, interventions and sampling timeframes) precluded meta-analysis.

Results

111 studies were included in the review.
One study provided good evidence that withdrawal of antimicrobial results in a reduction of identifiable resistance in potential pathogens in retail meat food for human consumption, and in humans, with credible effect sizes and time sequences.

There is also adequate evidence to conclude that limiting antimicrobial supplementation in food animals feed reduces the burden of antimicrobial resistance in animals, but insufficient evidence to quantify this effect (which may be specific to different antimicrobials at different doses, food animals and environments). Administration of one antimicrobial can induce resistance in an antimicrobial from a completely different class.

Conclusions

Limiting the use of antimicrobial supplementation for food animals is likely to reduce the presence of antimicrobial resistance in other food animals and humans. This may extend beyond the antimicrobial used to other antimicrobial classes.

More primary studies are necessary to strengthen the research evidence.

Background and Objectives

Antimicrobial resistance, Fact sheet N°194, Updated April 2015

a) Description of the issue

i. Disease burden and distribution across subgroups

Antimicrobial resistance is the direct consequence of antimicrobial use. The threat of antimicrobial resistance is not only a global health risk (estimated to be a direct cause of death that overtakes all cancers by 2050), but also a global economic threat of the same stature as terrorism.[1]

The use of antimicrobial agents in food animals has been proposed as a probable contributor to resistance.[1 2] Although the WHO estimates the health consequences of foodborne diseases[3], these estimates do not include the human health burden of antimicrobial-resistant foodborne diseases arising from antimicrobial agents use in food animals. The same antimicrobials are used for clinical treatment of humans.

The use of antimicrobials in food animals may be therapeutic (to treat a bacterial infection) or non-therapeutic (for growth promotion). Growth promotion benefit of antimicrobial agents arose from the observation that food animals grew bigger and faster when fed fermentation by-product waste during the production of streptomycin and penicillin in the 1940s in the United States. Antimicrobials were marketed worldwide as an additive to animal feeds shortly thereafter, even though the biological mechanism of, and the quantification for, this growth benefit was poorly understood.

Background on public health concern of use of antimicrobial agents in food animals

Public health concerns about the use of antimicrobial agents in food animals have been expressed for decades. Early public health concerns about the use of antimicrobial agents in food animals focused mainly on the use of antimicrobial agents in animal feeds for growth promotion. The United Kingdom government established the Netherthorpe Committee in 1960 to investigate whether use of antimicrobial agents in animal feeds constituted a danger to humans, followed by the Swann Committee in 1968 which concluded that administration of antimicrobial agents to food animals poses hazards to human and animal health from antimicrobial resistance.[4]

Since then, many scientific, regulatory, and professional organisations elsewhere in the world have deliberated on the problem.

The use of fluoroquinolones and third-generation cephalosporins for food animals in the 1990s, particularly as mass medications, marked a new wave of concern. The WHO Consultation on Medical Impact of the Use of Antimicrobials in Food Animals concluded that antimicrobials in food animals lead to selection of antimicrobial resistance which may be transmitted to humans, despite considerable uncertainty about the magnitude of the issue.[5]

WHO has escalated the concern over the next two decades.

Rationale for proposed WHO Guideline

The potential concerns are that antimicrobials used in food animals:

1. are widely used in humans;
2. may result in resistant bacteria in
   - food animals; and
   - humans.

This has resulted in calls for a WHO Guideline to preserve the long-term effectiveness of antimicrobials critically important for human medicine.

b) Objectives & Purpose

ii. Goal of the proposed WHO Guideline:

to preserve the effectiveness of antimicrobial agents critically important for humans.

iii. Purpose of the systematic review and the summary of evidence:

to find the evidence to test the hypotheses that *specific reductions of antimicrobial uses in food animals are necessary to reduce the prevalence of such resistance in food animals and in humans*.

It was commissioned to support the development of proposed WHO Guideline on use in food animals of critically important antimicrobial agents for human medicine, in the light of the WHO Global Action Plan (GAP) for combatting antimicrobial resistance, particularly the One Health work stream of the WHO GAP.

There are some assumptions arising from widespread consensus among the scientific community for the following.

1. Are antimicrobial agents used in food animals related to antimicrobial agents used to treat human?

   Antimicrobial agents are widely used in food animals and some antimicrobial agents used in food animals are identical to, or closely related to, antimicrobial agents used to treat humans.

2. Does the use of antimicrobial agents in food animals result in antimicrobial resistance in food animals?

   The use of antimicrobial agents in food animals results in selection and dissemination of antimicrobial-resistant bacteria in food animals.

3. Are antimicrobial resistant bacteria and antimicrobial-resistant determinants (such as transmissible plasmids carrying antimicrobial-resistant genes) transmitted from food animals to humans?

   Human pathogenic (e.g., Salmonella *and* Campylobacter*) and commensal (e.g.,* Escherichia coli, enterococci*) bacteria, including bacteria with antimicrobial resistant determinants, are transmitted to humans through food and, to a lesser extent, by direct animal contact.*

4. Are human infections with antimicrobial-resistant bacteria associated with more severe human health consequences compared to human infections with antimicrobial-susceptible bacteria?

   Infections with antimicrobial-resistant bacteria, including antimicrobial-resistant foodborne bacteria (such as non-typhoidal Salmonella *and* Campylobacter*), are associated with more severe human health consequences, including treatment failures, increased or longer hospitalisations, and prolonged illnesses, compared with infections with antimicrobial-susceptible bacteria.*

However there is less consensus on the *extent* to which antimicrobial use in food animals contributes to antimicrobial resistance in humans.

iv. Objective of the systematic review and summary of evidence:

The commission for the systematic review and summary of evidence was to provide a rapid systematic review of two questions, formulated into PICO format to break the questions into their component parts of

P Population
I Intervention
C Comparator
O Outcomes

PICO Question One:

For food animal populations of any age in any setting, does a limitation compared to not having that limitation of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in food animal populations?

PICO Question Two:

For human populations of any age in any setting, does a limitation compared to not having that limitation of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in human populations?

Population [P] =

- PICO question one: food animals of any age [S] in any setting.
- PICO question two: humans of any age [S] in any setting i.e. community or healthcare facility

Intervention [I] =

- a limitation of use of antimicrobial agents(s) at any time period [T] in food animals whereby a limitation is defined as any level of restriction. A limitation is defined as any level of restriction up to and including a complete cessation (i.e., following a prohibition on use). The restriction of use of antimicrobial agents(s) may be applied either alone or in combination with other antimicrobial agents(s). The limitation may be voluntary or imposed by regulation, policy or the marketplace.

Comparator [C] =

- not having that limitation of use antimicrobial agent(s) at any time period [T] in food animals

Outcome [O] =

- PICO question one: the presence of antimicrobial resistant bacteria and/or antimicrobial-resistant genetic elements in food animal populations
- PICO question two: the presence of antimicrobial resistant bacteria and/or antimicrobial-resistant genetic elements in human populations

c) Framework

Conceptual map

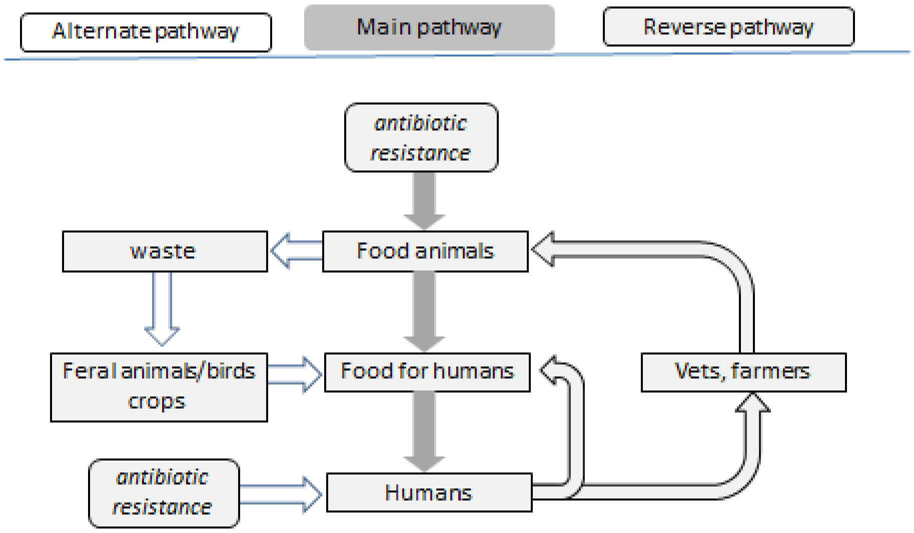


Figure 1 **A conceptual map of the sources of antimicrobial resistance into humans from food animals, and alternative and reverse pathways (adapted and simplified from Phillips 2004)**

Antimicrobial resistance follows after antimicrobial use. Thus the main pathway for antimicrobial resistance follows its use in animal foodstuffs. This may result in resistance reaching humans after directly entering the household for consumption.

This may also occur in alternative ways, such as the resistance contaminating the local environment (through waste) and being transferred to humans directly, or by vectors such as rodents, or into crops.

The picture might be complicated by *reverse pathways* such as resistance that developed in humans by their therapeutic use of antimicrobials being transferred to food animals.

Evidence in humans suggests that the converse is also true – that *not* using antimicrobials leads to the dissipation of resistance.[Cottesloe 2010]

One problem for estimating the effects of antimicrobials on resistance in any environment is the measurement of resistance.

This is because we might expect the effect of antimicrobials to influence the numbers of bacteria as follows (Fig 2)

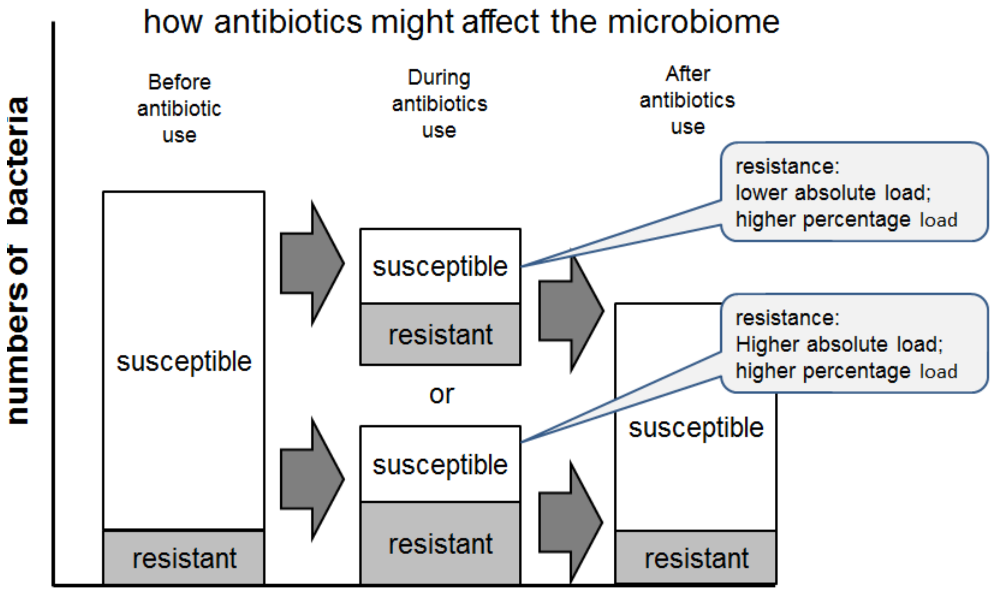


Figure 2 **Anticipated effects of antimicrobials on numbers of susceptible and resistant bacteria**

Before exposure to antimicrobials we might expect to see a baseline small proportion of resistant bacteria. During exposure, the total proportion of bacteria in the host's microbiome might be expected to be reduced by the lethal effect of the antimicrobial. However the proportion of bacteria resistance to the antimicrobial might increase because in an environment of antimicrobial, resistance bacteria will have a selective advantage. The population of resistant bacteria might increase or decrease from before antimicrobials.

Note that there are two variables that influence the outcomes: antimicrobials increase the proportion of bacteria that are resistant; and they also reduce the total population of bacteria. The outcome of interest to the community is the total load of resistant bacteria, but measuring this requires knowledge of both the *proportion* of resistant bacteria and the *total bacterial population*. Studies that measure only the proportion of resistant bacteria without an estimate of the total bacterial population might provide a misguided outcome measure. This means we might need to consider two types of results, Fig 3.

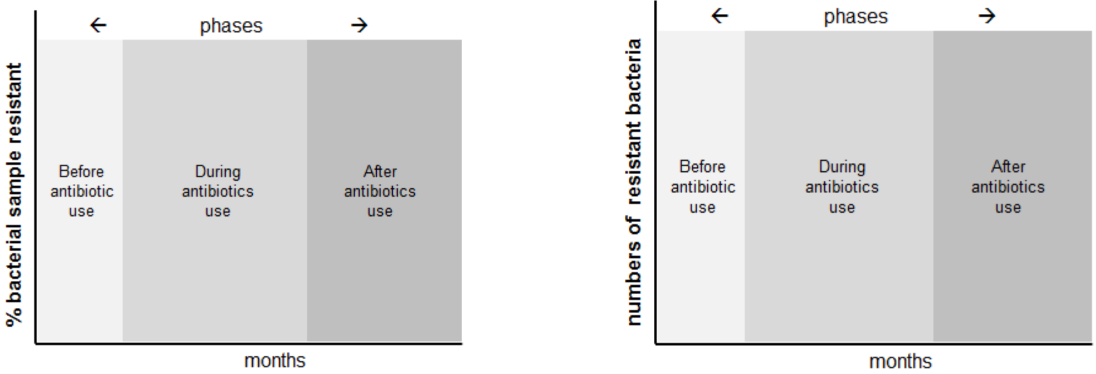


Figure 3 Effect of antimicrobials on numbers of *proportions* and *absolute numbers* of susceptible and resistant bacteria

If antimicrobials are stopped, then the total population of bacteria might increase again, but in time the resistant bacteria would be expected to decrease as the competitive environment no longer favours resistance – although the population of resistance bacteria might decrease or increase.
This means research studying the problem can report the relevant changes in microbiome as a change in either the:

1. *proportion* of resistant bacteria; *or*
2. *absolute load* of resistant bacteria.

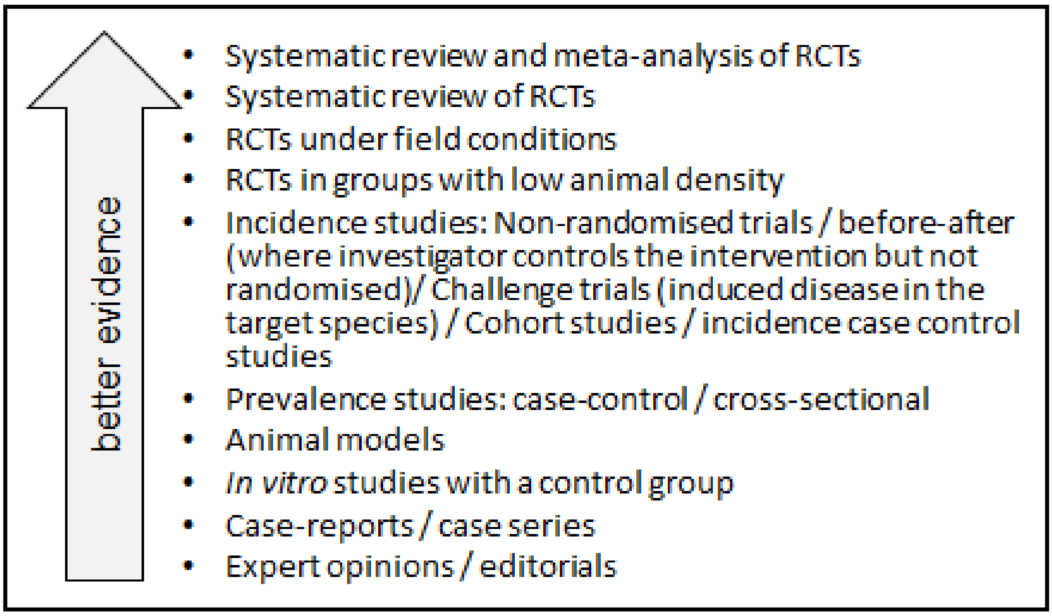
Rapid systematic reviews of the literature

Normally systematic reviews to answer questions required for WHO guidelines can take two years to develop. However this question needed a far faster approach. For example one rapid systematic review was undertaken in 5 weeks for a focussed review of a series of randomised trials (on neuraminidase inhibitors for influenza).[6]

However this topic area is more complicated, with a variety of study types including experimental and observational studies; an enormously wide approach to analysing and reporting research; many different animals; many different antimicrobials under consideration; and many different environments.

Our approach to this rapid systematic review

The ideal study type to answer these two questions would be experiments in the form of randomised trials. The supplementary antimicrobial supplied to animals in their feed was experimentally stopped, and changes in resistance among proximal animals and humans recorded. Because experimental evidence was scant, we also had to include both challenge trials and observational studies.
A hierarchy of evidence for animal studies places challenge trials on par with non-randomised (controlled) trials and cohort studies, Figure 4.



- Systematic review and meta-analysis of RCTs
- Systematic review of RCTs
- RCTs under field conditions
- RCTs in groups with low animal density
- Incidence studies: Non-randomised trials / before-after (where investigator controls the intervention but not randomised)/ Challenge trials (induced disease in the target species) / Cohort studies / incidence case control studies
- Prevalence studies: case-control / cross-sectional
- Animal models
- *In vitro* studies with a control group
- Case-reports / case series
- Expert opinions / editorials

Figure 4 **A hierarchy of evidence for animal studies (modified from Sargeant 2014[7])**

Notes:

Ecological research study type in medical research means *observational research based in different environments* (usually comparing countries or large regions which provide differing exposure to the outcome of interest), and is subject to a number of biases; in animal research it refers to *communal sampling of several individual animals* (for example, the faeces of cattle sampled from a herd to determine antimicrobial resistance).

Challenge trials are experiments in which a population of animals is deliberately inoculated with resistant bacteria, for example, to enable experiments that randomly assign animals to either an intervention or control methods of reducing resistance. Theoretically this design is free of many forms of bias, like RCTs. However, challenge trials are considered less strong evidence because of the artificial arrangement than RCTs with natural exposure to disease.[7] It is the design of Ojeniyi 1989.

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The quality of research in this area is difficult to assess. Not only are research methods often non-standardised (investigative techniques used are rarely duplicated); but also reported in an unstructured manner, creating difficulties in following the method employed.

Methods

Timeline

Conventional systematic reviews typically require both considerable resources, and between six months and 2 years to complete. Therefore, in order to enable reviewers to provide evidence syntheses on shorter timelines, rapid systematic review methodologies have been developed. These methodologies allow completion of evidence syntheses within 1-12 months, with typical completion ranging from 3-9 months.[8-10]

Staff

The present evidence synthesis is a result of collaboration by the following individuals:

- Systematic review methodologist (AS, CDM, PG)
- Information specialist (JC)
- Content expert (DT)
- Biostatistician (EB)
- Staff experienced in conducting systematic reviews (PC, RR, OB, MB)

In the initial stages (during preparation of the validation set of articles for the literature searches, discussed below), advice and guidance was also provided by members of the WHO Advisory Group. However, once the work begun, no further advice or guidance were sought (other than feedback on the draft report), in order to minimise the potential for biasing the results of the evidence synthesis.

Search strategy

a) Validation set of references for database searches

Before undertaking literature searches, we generated a *validation set* of references. This is a set of references known to be relevant to a question of interest and which needs to be identified by a literature search. *Validation sets* are used to test whether a literature search is robust (ie misses a minimum number of relevant articles).

To generate the validation set, we:

1. *Undertook forward and backward citation searches*
2. *Repeated and updated search strategy in a recent relevant report on the subject*
3. *Requested known relevant studies from the WHO Advisory Group*

i. Forwards and backwards citation searches

The forwards and backwards citation searches were conducted in May 2016. This involved:

a. Identifying existing, recent literature reviews and government reports, including
    - The report from Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) (1999) “The use of antibiotics in food-producing animals: antibiotic-resistant bacteria in animals and humans.” (relevant to answering PICO1)
    - Wong et al (2012) “The transfer of antibiotic resistance from food to humans: facts, implications and future directions.” (relevant to answering PICO2)
b. Examining their reference lists, to generate a list of relevant primary studies: 8 potentially relevant references were identified
c. Doing forward- and backward-citation searches of the 8 relevant primary studies (i.e. identification of references that subsequently cited these studies, as well as identification of those that they themselves cited, respectively), in order to identify additional studies which were relevant: 389 references were identified
d. These 389 references were screened by title/abstract for inclusion by two authors: 17 references were identified for full text review
e. The full-text review yielded 9 studies for inclusion in the validation set

ii. Repeat and update of search strategy in a recent relevant report on the subject

The UK’s *Review on Antimicrobial Resistance* December 2015, chaired by Jim O’Neill, published a report *Antimicrobials in agriculture and the environment: reducing unnecessary use and waste*, which contained a search strategy that we replicated, updating it to 26 May 2016.

1. The repeated and updated search yielded 312 references

2. These were screened on title/abstract for relevance to PICO questions by one author: 45 references were identified for full text review
3. The full-text review yielded 10 studies for inclusion in the validation set

iii. Request for known relevant studies from the WHO Advisory Group

Members of the WHO Guidelines Group were requested to provide the reviewers with key known relevant studies. The group provided the reviewers with 12 references, and two endnote libraries (one containing 34 references, one containing 59 references) for potential inclusion.

- All references were screened in full-text for inclusion
- This yielded 7 studies for the validation set

The references obtained through forward and backward citation searches, the repeat and update of search strategy in the O'Neill report, and from the WHO Guidelines Group were amalgamated and de-duplicated. The resultant *validation set* is listed in Table 1, below.

Table 1 Validation set of articles for the literature searches

1 Aarestrup, F. M. (1995). Occurrence of glycopeptide resistance among Enterococcus faecium isolates from conventional and ecological poultry farms. Microb Drug Resist, 1(3), 255-257. doi: 10.1089/mdr.1995.1.255

2 Aarestrup, F. M., et al (2001). Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother, 45(7), 2054-2059. doi: 10.1128/aac.45.7.2054-2059.2001

3 Alali, W. Q. et al (2008). Longitudinal study of antimicrobial resistance among Escherichia coli isolates from integrated multisite cohorts of humans and swine. Appl Environ Microbiol, 74(12), 3672-3681. doi: 10.1128/aem.02624-07

4 Alali, W. Q. et al (2009). Relationship between level of antibiotic use and resistance among Escherichia coli isolates from integrated multi-site cohorts of humans and swine. Prev Vet Med, 90(3-4), 160-167. doi: 10.1016/j.prevetmed.2009.05.018

5 Alali, W. Q. et al (2010). Assessing the similarity of antimicrobial resistance phenotypes among fecal Escherichia coli isolates from two aggregated occupational cohorts of humans versus swine using cluster analysis and multivariate statistics. Prev Vet Med, 94(1-2), 77-83. doi: 10.1016/j.prevetmed.2009.11.014

6 Bauer-Garland, J. et al (2006). Transmission of Salmonella enterica serotype Typhimurium in poultry with and without antimicrobial selective pressure. J Appl Microbiol, 101(6), 1301-1308. doi: 10.1111/j.1365-2672.2006.03036.x

7 Borgen, K. et al (2000). Continuing high prevalence of VanA-type vancomycin-resistant enterococci on Norwegian poultry farms three years after avoparcin was banned. Journal of Applied Microbiology, 89(3), 478-485. doi: 10.1046/j.1365-2672.2000.01137.x

8 Dorado-Garcia, A. et al (2015). Dose-response relationship between antimicrobial drugs and livestock-associated MRSA in pig farming. Emerg Infect Dis, 21(6), 950-959. doi: 10.3201/eid2106.140706

9 Dutil, L. et al (2010). Ceftiofur Resistance in Salmonella enterica Serovar Heidelberg from Chicken Meat and Humans, Canada. Emerg Infect Dis, 16(1), 48-54. doi: 10.3201/eid1601.090729

10 Getachew, Y. et al (2013). Genetic Variability of Vancomycin-Resistant Enterococcus faecium and Enterococcus faecalis Isolates from Humans, Chickens, and Pigs in Malaysia. Appl Environ Microbiol, 79(15), 4528-4533. doi: 10.1128/aem.00650-13

11 Gupta, A. et al (2004). Antimicrobial resistance among Campylobacter strains, United States, 1997-2001. Emerg Infect Dis, 10(6), 1102-1109. doi: 10.3201/eid1006.030635

12 Herrero-Fresno, A. et al (2016). Apramycin treatment affects selection and spread of a multidrug-resistant Escherichia coli strain able to colonize the human gut in the intestinal microbiota of pigs. Veterinary Research, 47. doi: 10.1186/s13567-015-0291-z

13 Klare, I. et al (1999). Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microbial Drug Resistance-Mechanisms Epidemiology and Disease, 5(1), 45-52. doi: 10.1089/mdr.1999.5.45

14 Levy, S. B. (1978). Emergence of antibiotic-resistant bacteria in the intestinal flora of farm inhabitants. J Infect Dis, 137(5), 689-690.

15 Levy, S. B. et al (1976). Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. N Engl J Med, 295(11), 583-588. doi: 10.1056/nejm197609092951103

16 Looft, T. et al (2012). In-feed antibiotic effects on the swine intestinal microbiome. Proceedings of the National Academy of Sciences of the United States of America, 109(5), 1691-1696. doi: 10.1073/pnas.1120238109

17 Mathew, A. G. et al (1999). Multiple antibiotic resistance patterns of Escherichia coli isolates from swine farms. Appl Environ Microbiol, 65(6), 2770-2772.

18 Ojeniyi, A. A. (1989). Direct transmission of escherichia-coli from poultry to humans. Epidemiology and Infection, 103(3), 513-522.

19 Park, Y. K. et al (2012). Prevalence and antibiotic resistance of mastitis pathogens isolated from dairy herds transitioning to organic management. J Vet Sci, 13(1), 103-105.

20 Public Health Agency of Canada (PHAC) (2007) “Salmonella Heidelberg – Ceftiofur-Related Resistance in Human and Retail Chicken Isolates.” PDF available at: http://www.phac-aspc.gc.ca/cipars-picra/heidelberg/heidelberg-eng.php

21 Scott, H. M. et al (2005). Patterns of antimicrobial resistance among commensal Escherichia coli isolated from integrated multi-site housing and worker cohorts of humans and swine. Foodborne Pathog Dis, 2(1), 24-37. doi: 10.1089/fpd.2005.2.24

22 Siegel, D. et al (1975). Human therapeutic and agricultural uses of antibacterial drugs and resistance of the enteric flora of humans. Antimicrob Agents Chemother, 8(5), 538-543.

23 Unicomb, L. E. et al (2006). Low-level fluoroquinolone resistance among Campylobacter jejuni isolates in Australia. Clin Infect Dis, 42(10), 1368-1374. doi: 10.1086/503426

24 van den Bogaard, A. E. et al (2001). Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. J Antimicrob Chemother, 47(6), 763-771.

25 Vinayagamoorthy, T. (1987). Mobilization of antibiotic resistance genes among farm animals and human hosts in a developing country (Sri Lanka). Singapore Med J, 28(2), 134-139.

26 Young, I. et al (2009). Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: a systematic review and meta-analysis. Epidemiology and Infection, 137(9), 1217-1232. doi: 10.1017/s0950268809002635

b) Database searches

The validation set was subsequently used to test the search strategy, with two modifications:

- Studies appearing to be of ecological design were excluded from the validation set, because the better-than-expected yield of higher level evidence meant we could focus on studies with lower bias: Unicomb 2006; Gupta 2004; Alali (2008, 2009, 2010); Getachew 2013; Scott 2005.[1]

- Studies which lacked an abstract were also excluded from the validation set because it was decided that they were likely to be poorer quality: Aarestrup 1995, Levy 1978, Vinayagamoorthy 1987, and PHAC (2007).

These exclusions yielded a validation set of 15 articles.

Two separate searches were built for PubMed. The first search searched for studies addressing antimicrobial resistance among food animals (PICO question 1). The second searched antimicrobial resistance between food animals and humans (PICO question 2). We pooled the yield from the two searches and then checked this against the validation set, adjusting the search strategy to ensure all the articles in the validation set were found by the search, when it was deemed sufficiently robust to serve as our final search strategy.

The search strategy was then modified for two further databases: Embase and Web of Science. This required standard conversion practices, such as changing MeSH terms into Emtree terms; and removing Subject Terms from the Web of Science search since it does not use controlled vocabulary.

The full search strategies are provided in Appendix 1.

Other sources of evidence

i. Forward citation search on Dutil 2010

Due to the paucity of evidence identified for answering PICO question 2, we undertook a forward citation analysis on one of the most pertinent studies identified for answering this question – Dutil 2010.

- The forward citation search yielded 101 references
- These references were de-duplicated against the studies already identified in the evidence synthesis, yielding 71 references
- 71 references were screened by Title/Abstract by one author
- 66 references were excluded, yielding 5 references for full text review
- The 5 references were full text reviewed by another author, resulting in one additional inclusion: Hiki 2015

ii. Grey literature search

Cochrane Handbook recommends that grey literature be included in systematic reviews that aim to include randomised trials only, in order to increase the comprehensiveness of the search and decrease the potential for publication bias.[11] However, as the present review included a broader range of study types than randomised trials, and was additionally very time constrained, grey literature was not specifically searched. Where grey literature was identified during the search, however, it was considered for inclusion against the same inclusion/exclusion criteria as all other evidence sources identified – i.e. grey literature was not specifically excluded on the grounds of being grey literature.

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[1] The literature searches nevertheless picked up the majority of these references due to their content – Alali 2008, Alali 2009, Alali 2010, Getachew 2013, Scott 2005. The searches did not pick up Gupta 2004 or Unicomb 2006. It needs to be emphasised here that although these studies were not used as part of the validation set (i.e. for search purposes), every single study in the validation set (n=26) was full text reviewed for inclusion in the evidence synthesis.

iii. References from included studies (hand searches)

Although the searches identified a number of studies comparing the effect of using antimicrobial versus not using antimicrobial, considerably fewer studies were identified where stopping antimicrobial was compared to continuing antimicrobial. Therefore, reference list of Khatchatryan 2004 (included in PICO 1) was hand searched for other relevant references.

- A list of 57 references contained in Khatchatryan 2004 was screened on title/abstract for relevance to the present evidence synthesis
- 4 potentially relevant references were identified, and read in full text
- 2 references were included in the evidence synthesis: Rollins 1976, and Smith 1975

iv. Contacting authors

Cochrane Handbook recommends that investigators contact study authors in cases where the investigators are not able to extract all of the relevant information from the published study.[11] However time constraints precluded this lengthy step.

v. Utilising content experts

We included a content expert for this review because up to 24% of relevant evidence may be missed by failing to do so.[8] In addition (see above), members of the WHO Guidelines Group were requested to provide us with known key relevant articles. These totalled 12 individual references. Australian content experts also provided the reviewers with two endnote reference libraries (one containing 34 references, one containing 59 references). All of these references were read in full for inclusion in the evidence synthesis.

At the draft report review stage, the WHO Advisory Group provided 1 further reference for potential inclusion in the evidence synthesis. This reference was read in full for inclusion in the evidence synthesis.

Search limits

There were neither language nor date restrictions on the search to avoid publication bias.[8]

Inclusion/exclusion criteria

In order to estimate the likely volume and type of evidence existing in the literature regarding the two PICO questions, the first 100 references identified in the literature search were screened. The screen suggested that:

- there will be considerably more evidence pertaining to PICO 1 question than to PICO 2 question, *and*
- that the existing evidence for PICO 1 question will be of higher study design (e.g. systematic reviews, RCTs, cohort studies) than for PICO 2.

Therefore, the inclusion/exclusion criteria adopted for the two PICO questions were as follows:

PICO 1: For food animal populations of any age in any setting, does limitation compared to not having that limitation of use of antimicrobial agents in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in food animal populations?

- Inclusion: Relevant to answering the PICO1 question;
- Included study types: studies that were likely to be: reviews (systematic, literature) RCTs, challenge trials, controlled trials, or cohort studies;

- Excluded study types: studies that were certain or probable to be: case-control, interrupted time series, before and after, cross-sectional, ecological;
- No date or language restriction was used;
- No restriction on the basis of ready accessibility of literature was used

PICO 2: for human populations of any age in any setting, does limitation compared to not having that limitation of use of antimicrobial agents in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in human populations?

- Inclusion: Relevant to answering the PICO2 question;
- Included study types: studies that were certain or probable to be: reviews (systematic, literature), RCTs, challenge trials, controlled trials, cohort studies, case-control, interrupted time series, before and after;
- Excluded study types: studies that were certain or probable to be: cross-sectional, ecological;
- No date or language restriction was used;
- No restriction on the basis of ready accessibility of literature was used

Completeness check on the results of the literature search

We had initially planned to use two libraries of references on antimicrobial resistance as a 'completeness check' on the results of the literature search:

- Keep Antibiotics Working (2010) Significant science on antibiotic resistance: an annotated bibliography.
- PEW Charitable Trusts (2014) Bibliography on Antibiotic Resistance and Food Animal Production. Scientific Studies (1969-2014)

We were unable to perform this check because of time constraints. However, the Cochrane Handbook does not mandate a completeness check, and in any case this may have been unnecessary because of the validation check (which effectively undertakes this upfront). [11]

Search results and screening of the identified literature

Searches of PubMed, Embase and Web of Science yielded 7023 references. Additional records identified through other sources yielded 132 references. On amalgamation of both sets of references and de-duplication, 3709 references remained, and underwent title/abstract screen.

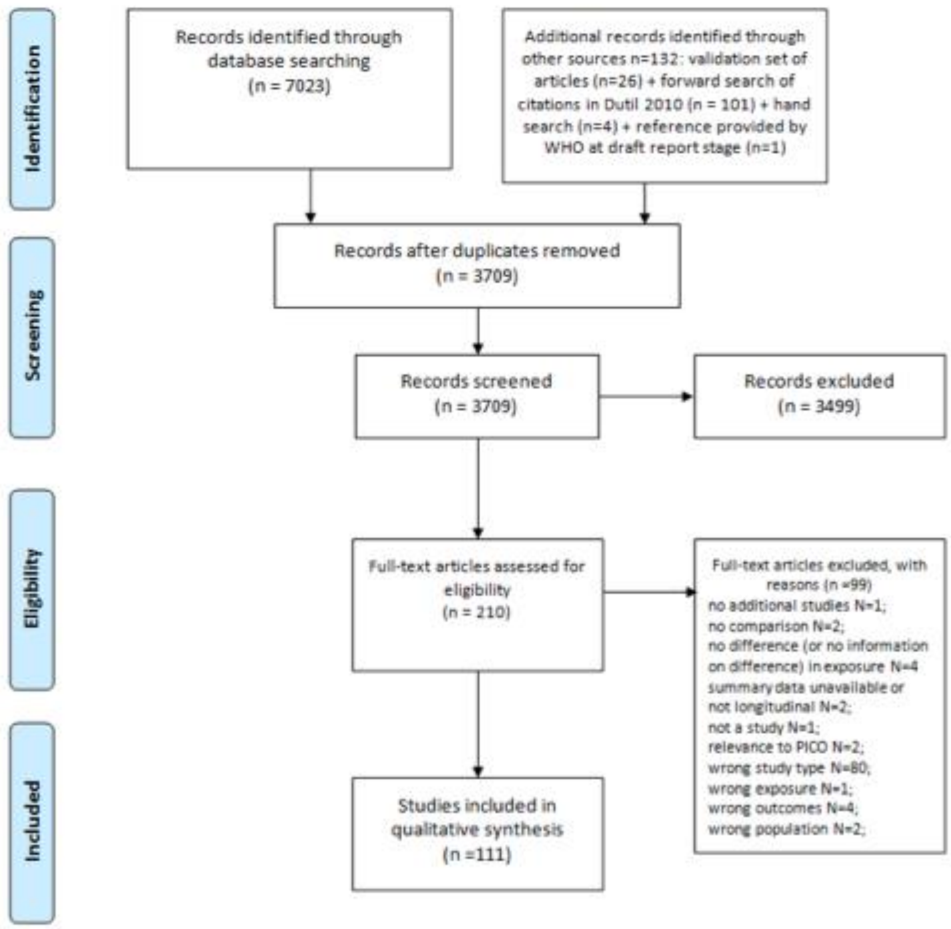
Cochrane handbook recommends that it is desirable that more than one author assesses the titles and abstracts for inclusion.[11] However, due to time constraints, rapid systematic reviews frequently have only a single reviewer do study screen.[10] In endeavouring to balance both time constraints and methodological robustness, the following method was adopted in the present review: all 3709 references were screened on title/abstract/keywords by one author, for relevance to PICO question 1 or PICO question 2. A random sample of 10% of the references (n=371) were also screened by another author. The two authors then met for identification of discrepancies in inclusion / exclusion decisions – as a result of this screen, 9 previously excluded references were included for full text review.

210 articles were full-text reviewed for inclusion by four authors working in two pairs; each individual reference underwent independent full-text review by two reviewers. Reviewers labelled each article as 'include,' 'exclude' or 'query.' Following an assessment of a batch of approximately 50 articles, each pair of reviewers met to discuss their decisions and resolve differences of opinion. This was repeated until all articles were full-text reviewed by two reviewers. In line with standard systematic review methodology, where the two reviewers came to different conclusions regarding an article, the discrepancy was resolved by discussion

between the two reviewers. Where this did not resolve the discrepancy, the article was referred to a third reviewer for arbitration.

On completion of the full-text review, 99 references were excluded and 111 were included in the evidence synthesis.

PRISMA Flow Diagram


Identification
Screening
Eligibility
Included
Records identified through database searching (n = 7023)
Additional records identified through other sources n=132: validation set of articles (n=26) + forward search of citations in Dutil 2010 (n = 101) + hand search (n=4) + reference provided by WHO at draft report stage (n=1)
Records after duplicates removed (n = 3709)
Records screened (n = 3709)
Records excluded (n = 3499)
Full-text articles assessed for eligibility (n = 210)
Full-text articles excluded, with reasons (n =99)
no additional studies N=1;
no comparison N=2;
no difference (or no information on difference) in exposure N=4
summary data unavailable or not longitudinal N=2;
not a study N=1;
relevance to PICO N=2;
wrong study type N=80;
wrong exposure N=1;
wrong outcomes N=4;
wrong population N=2;
Studies included in qualitative synthesis (n =111)

After : Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Data extraction

Two data extraction forms were prepared: a Table of Included Studies form, and the Summary of Findings form.

Both forms were test piloted on four articles by seven of the authors, and were amended in light of feedback received.

The forms were then utilised to extract information on each included study (into the Table of Included Studies form) and to extract data from each study (into the Summary of Findings Form). Extraction was conducted by five authors into the forms, with a check on each extraction by another author.

Risk of bias / quality appraisal

The included studies were not formally assessed for quality, but rather they were categorised according to the hierarchy of evidence proposed in Sargeant 2014 (see Discussion section). Tables of Results for studies included in the evidence synthesis include information on limitations and provide additional comments where applicable.

Meta-analysis

The identified evidence evinced considerable heterogeneity with regard to study design, interventions evaluated, settings, antimicrobials and time points at which outcomes were measured. Due to this heterogeneity, meta-analysis was not possible. Therefore, the results are presented as a narrative.

Review of draft

First draft of the report was reviewed by the WHO Advisory Group on 1 September 2016. Feedback and suggestions provided at that stage have been incorporated into the present version of the review.

Results for PICO question 2

The included studies are tabled.

Table 2 **Characteristics of included studies with eligible designs**

Controlled trials

Study ID	**Method**	**Groups**	**Exposure**	**Result and Limitations for required Questions**
Ojeniyi 1989 Denmark	MIC on sensitivity test agar. Groups defined by level of contact with fowl with experimentally inoculated with antibiotic resistant *E coli*. Comparison: fowl and humans remote from the experiment.	A. Workers at university experimental pen (n=4) B. Birds in university experimental pen (n=36)	High	All farm attendants at commercial farm had detectable resistance gene by 2 months after inoculation. 2 of 4 attendants at university farm had resistance by 4 months, and 3 of 4 by 8 months. See Figure R1. Insufficient follow-up (8 days) to see if there is a decline in resistance with time in animals or humans.
		C. Workers at other university pens (n=8) D. Workers elsewhere on university farm (n=5) E. Workers at commercial experimental pen (n=3) F. Birds in commercial experimental pen (n=16) G. Workers elsewhere on commercial farm (n=2)	Medium	
		H. Residents not on farms (n=4) I. Birds kept in villages, not on farms (n=24)	Low	

Cohort studies

Study ID	Method	Groups	Exposure	Result and Limitations for required Questions
Alali 2008a, 2009a, 2010a United States	MIC on sensitivity test agar.	Swine workers Non-swine workers	Overall levels of resistance were low (0 – 25% of isolates). There was no significant association between amoxicillin dose or tetracycline dose and resistance levels in either swine workers or non-swine workers. In non-swine workers there was a significantly higher level of resistance in those who used ciprofloxacin, but not in swine workers. See Fig R2 below	Methods for calculating antibiotic dose in humans and animals not clear. No information about resistance levels on ceasing antibiotics. See Table 3 in the paper. Dose-response relationship between dose of antibiotic consumed (MMD – mean monthly dose) and resistance in both swine workers and non-workers.
Dorado-Garcia 2015 The Netherlands	Agar plates	Pigs Human farm residents	There was a significant dose-response relationship over all farms between dose of antibiotic and MRSA, with a 16% increase in odds for a 2-fold doubling of dose in pigs, and a 1.2% increase in odds for a 2-fold doubling of dose in humans. See Fig R3 below	See Figure 4 in the paper for dose-response relationship between dose of antibiotic fed to pigs and resistance in pigs and humans on farms. No information about resistance levels on ceasing antibiotics.
Levy 1976 United States	Agar plates	Tetracycline-fed chickens (50% of intake) Non-tet-fed chickens (50% of intake) Farm residents Neighbours Urban controls	Resistance observed in non-tet-fed chickens and farm dwellers 4 to 5 months after introduction of tet-feed in chickens. No difference between neighbours who ate eggs from tet-fed chickens and neighbours who ate eggs from non-tet-fed chickens.	See Table 1 in the paper for main result in farm dwellers. Not sure if can graph this easily? No information about resistance levels on ceasing antibiotics.

Kuhn 2005 Sweden, Spain, UK, Denmark	Agar plates	Humans Animals	Vancomycin-resistant enterococcci detected in very few samples from animal sources in Sweden where avoparcin banned, compared with up to 26% of samples in Spain. VRE detected in samples from human sources in all countries, with up to 90% in Spain, mostly non-animal source strains.	See Table 1 in Main paper for results. Ecological measures only – no individual sampling.

Interrupted time series

Study ID	**Method**	**Groups**	**Exposure**	**Result and Limitations for required Questions**
Dutil 2010 PHAC 2007 CIPARS 2016 Canada	Data from resistance monitoring programmes	Before, after ceasing ceftiofur – retail store meat Before, after ceasing ceftiofur – human isolates	No change seen in Ontario, but decrease in Quebec in retail meat. Increase in Saskatchewan after reintroduction of ceftiofur. Significant decrease in Ontario and Quebec in human resistant infections. See Figure R6.	Results -- see Figure 1 and the table in the paper Aggregated over year time frames. Small number of measures after reintroduction of ceftiofur.
Gupta 2004 United States	Data from resistance monitoring programmes	Before and after introduction of fluoroquinolones (1995-6) for use in poultry.	Odds ratio for resistance increased significantly to 2.5 times the 1997 (before fluoroquinolones) level by 2001. No significant trend in resistance to other antibiotics. See Figure R4.	See Table 2 in paper for human results. Only one measure before introduction of fluroquinolones. No information about resistance levels on ceasing antibiotics.
Nachamkin 2000 (Silberberg 2008) Data from Spain	NR	Before and after introduction of fluoroquinolones (1990) for use in poultry and livestock.	Resistance of *Campylobacter* to fluoroquinolones of clinical isolates in humans over time increased from less than 10% in human isolates before the introduction of fluoroquinolones to >80% by 1996 See Figure R5.	No information about resistance levels on ceasing antibiotics.

Summary of results for PICO question 2

The results from the highest quality study, Ojeniyi 1989, which was a challenge trial, (although not randomised), are displayed as a graph. It uses data adapted from Ojeniyi 1989 Table 4.

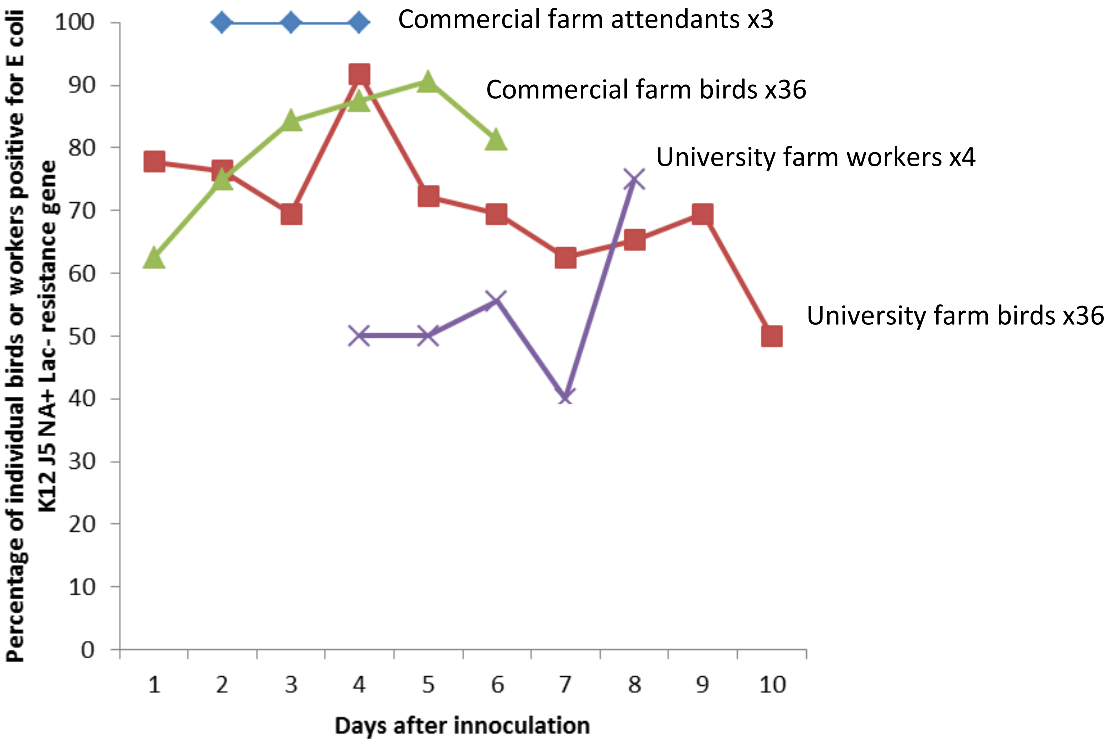
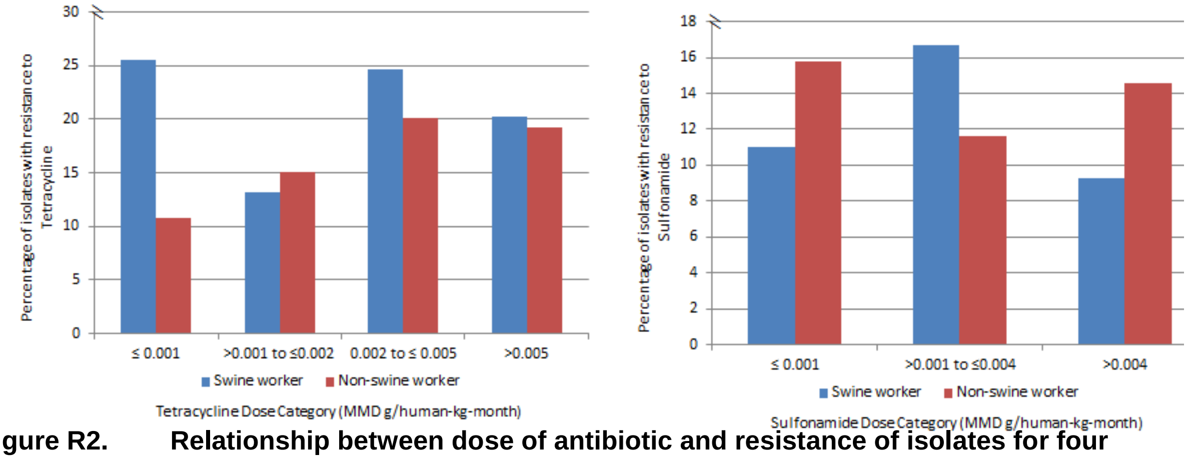


Figure R1 Percentage of individual birds or workers positive for *E coli K12 J5 NA+ Lac-* resistance gene following inoculation (data extracted from Fig of Ojeniyi 1989). Note that comparative animal (chicken in free-living nearly villages) and human controls (nearly villagers un-connected to the experimental or broiler farms) were all negative for resistance to nalidixic acid.

This suggests that after inoculation of chickens with an antibiotic resistance gene carried on an *e coli* species, the resistance

1 became widespread among most individual animals inoculated; and
2 was transferred to
    a. other animals in proximity; and
    b. humans in proximity
3 while other animals and humans remained free of the resistance.

This does not answer either PICO1 or PICO2 questions



Panel C – Tetracycline

Panel D – Sulfonamide

Figure R2. Relationship between dose of antibiotic and resistance of isolates for four antibiotics in humans. Redrawn from data in Alali 2009a.

The only statistically significant relationship from these data provided by the authors was an increase in the non-swine-workers with exposure to increasing concentrations of tetracycline (Panel C). In summary, there is no information here that helps us answer the research questions required by the WHO AMG team.

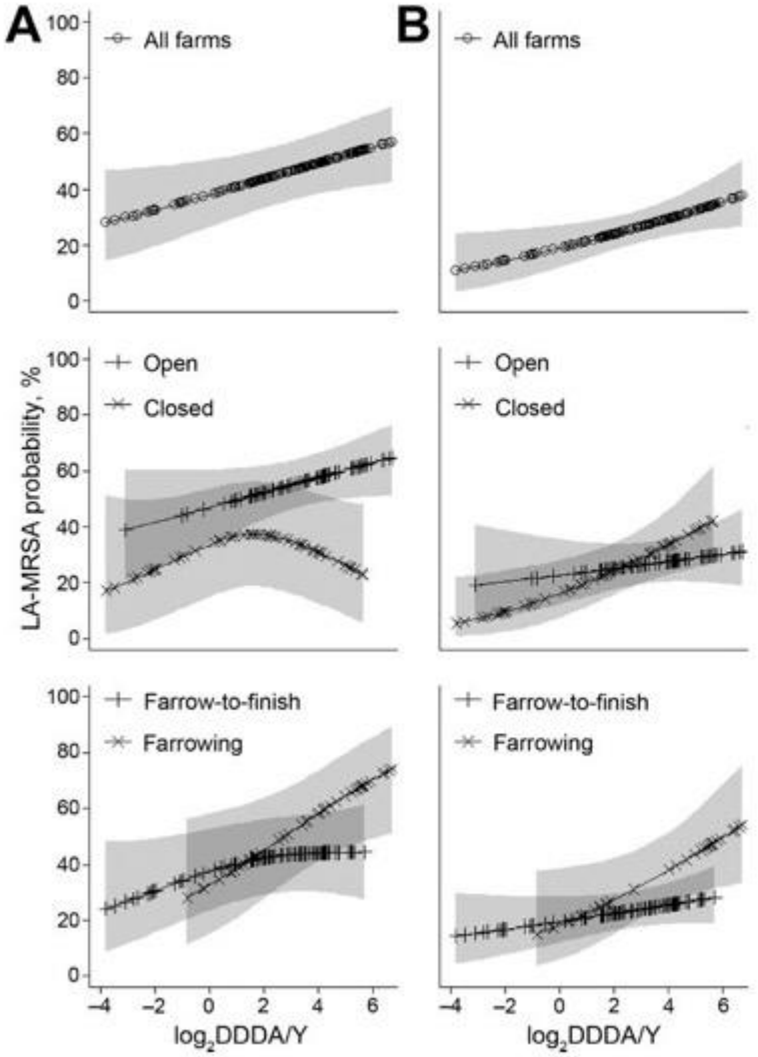


Figure 4. Dose–response relationships between antimicrobial use ($\log_2$ DDDA/Y) and livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) predicted probabilities in pigs (A) and humans (B), the Netherlands, 2011–2013. Splines were obtained from generalized additive mixed models with random intercepts for farms in the analysis for pigs and humans. Models accounted for the repeated measurements design and were adjusted for age group of pigs and for animal contact (i.e., hours worked) for humans. DDDA/Y was determined by dividing the total number of kilograms treatable with a single mass unit of the antimicrobial drug concerned, in accordance with the package insert information, by the average number of animal kilograms on the farm. Farms were defined as open when they received external supplies of gilts ≥1 time per year from at least 1 supplier and as closed when they received no external supply of gilts. p values and maximum-likelihood (ML) scores for the splines in the models for pigs: all farms ($p = 0.03$; ML 1433.5); open farms ($p = 0.09$; ML 991.3); closed farms ($p = 0.09$; ML 407.9); farrowing farms ($p = 0.02$; ML 438.5); farrow-to-finish farms ($p = 0.39$; ML 936.5). p values and ML scores for the splines in the models for humans: all farms ($p = 0.01$; ML 573.9); open farms ($p = 0.41$; ML 337.8); closed farms ($p = 0.01$; ML 229.9); farrowing farms ($p = 0.03$; ML 170.3); farrow-to-finish farms ($p = 0.17$; ML 398.2). DDDA/Y, defined daily dosages per animal per year; ML, maximum likelihood. Shaded areas indicate 95% CIs.

Figure R3. Modelled relationship between dose of antibiotic and livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA). Panel A pigs, Panel B humans. Taken from Dorado-Garcia 2015. (Note ‘gilt’ is a young female pig; a ‘farrow’ is one of a litter of pigs)

These data provide evidence for an association between exposure to antibiotics in animal feedstuffs and the development of resistance in both animals and humans in a dose-dependent manner. However it does not answer the evidence question posed by the Guidelines team, which is the reverse of this (does stopping antibiotics reduce antibiotic resistance?).

Data re-drawn from Gupta 2004 and Silbergeld 2008

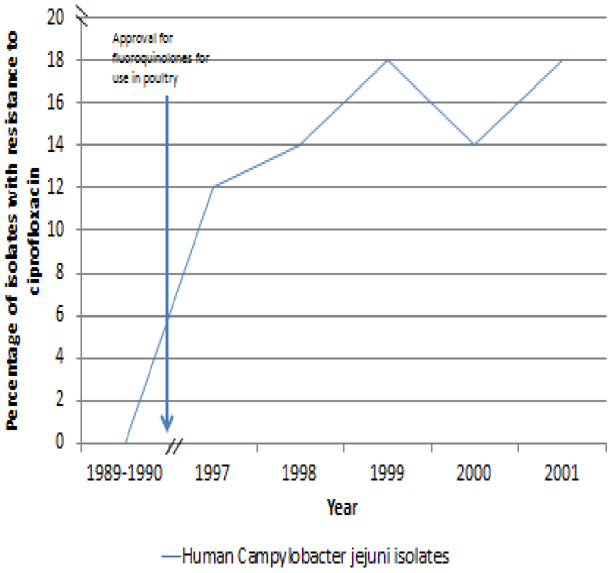


Figure R4. Resistance of *Campylobacter* to ciprofloxacin of clinical isolates in humans over time in the USA. Redrawn from Gupta 2004 (Fig 2).

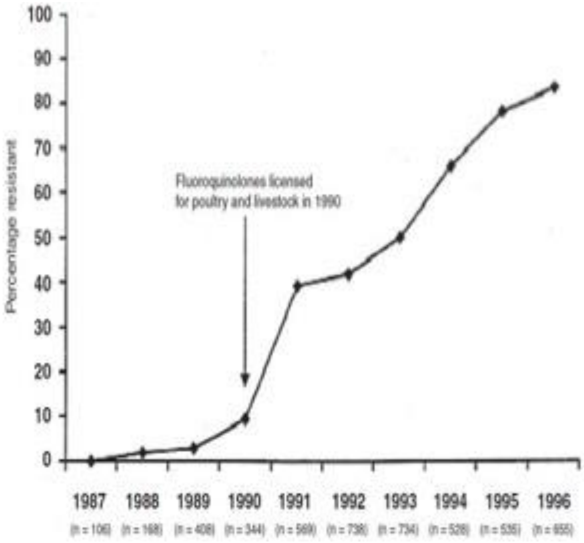


Figure R5. Resistance of *Campylobacter* to fluoroquinolones of clinical isolates in humans over time in Spain. Extract, Fig 3 from Silbergeld 2008.

Both these studies show that use of one antibiotic, with wide implementation in the animal food industry, is associated with an increase in the percentage of bacteria resistant to that antibiotic in humans.

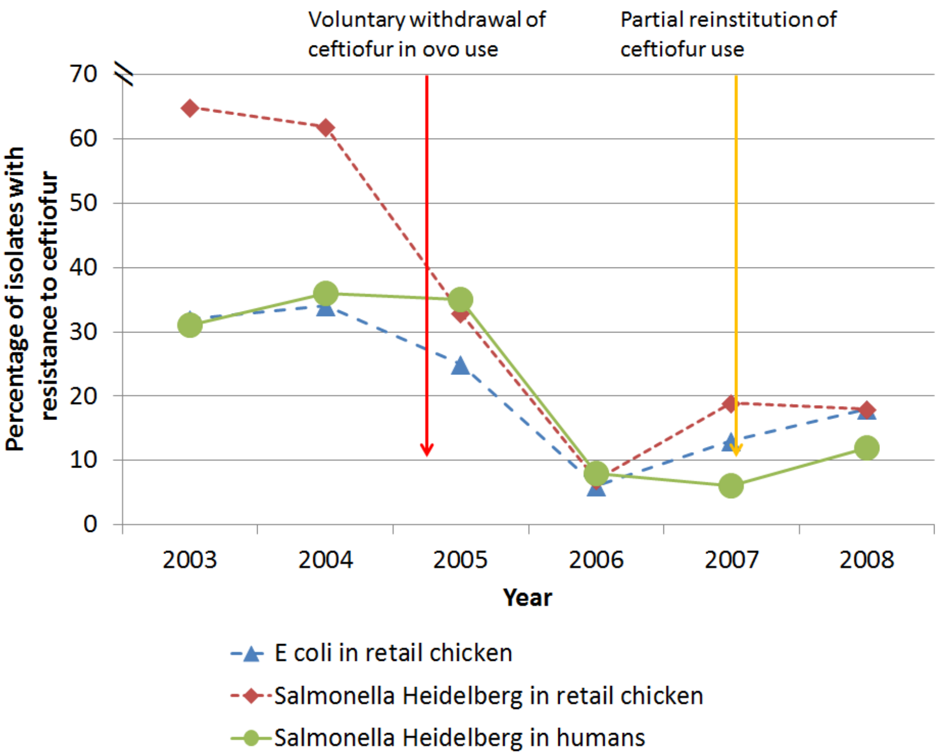


Figure R6. Resistance to ceftiofur over time in Quebec province, Canada. Redrawn from data in Dutil 2010.
This study shows how ceftiofur-resistant *Salmonella enterica* serovar Heidelberg isolated from retail chicken carcasses and clinical samples in humans varied with time during which ceftiofur was withdrawn and then re-introduced.

Because of the multiple time points, and the very strong effect size (near fourfold change), this evidence should be graded upwards. These data show:-

- an association between the resistance in animals fed antibiotics and resistance in clinical samples of humans;
- that withdrawal of the antibiotic in animal foodstuffs results in a reduction in resistance in animals used for human consumption;
- and in humans;
- re-introduction of the antibiotic in animal foodstuffs results in an increase in resistance in animals used for human consumption; and
- and in humans; and
- changes in resistance effects occur in humans *after* they appear in animals

The last point fits with the assumed flow of resistance in the chain (see Fig 1 in Background).
This is the strongest evidence to answer the required questions.
Accordingly we undertook a forwards- and backwards-citation search (see results of this search in the PRISMA flow diagram, p18).

Results for PICO question 1

The included studies are tabled, grouped by descending methodological rigour.

RCTs under field conditions								
Study ID / Location	**Exposure / Comparison**	**Approach / Design**	**Setting / Duration**	**Outcome Measure**	**Method**	**Group**	**Result**	**Limitations and Comments**
Agga 2015 (results) Agga 2014 (methods) United States	Antibiotic vs none, in presence/ absence of copper.	2 x 2 factorial randomised trial (N.B. was analysed as 4-arm trial)	Experimental farm / 5 weeks	Proportion of samples with resistance genes to tetracycline.	Gene copy numbers / gram.	A. Pigs fed control feed (with standard low dose copper supplement)	Increased tetA and bla$_{CMY\text{-}2}$ genes in CTC-treated groups. Copper did not produce this rise, hence might be recommended as an alternative to antibiotics.	No information about resistance levels after ceasing antibiotics.
						B. Pigs fed high dose copper		
						C. Pigs fed high dose copper and CTC		
						D. Pigs fed standard dose copper and CTC		
Alexander 2008; Mirzaagha 2011 Canada	Antibiotic vs none	6-arm cluster-randomised trial. antibiotic (5 different regimens), no antibiotic.	Experimental feedlot / 315 days	Proportion of animals with resistance.	CFUs on agar	A - E. Steers with 5 different antibiotic regimens in feed. antibiotic withdrawn and re-introduced.	Group fed combination of chlortetracycline and sulfamethazine had highest resistance levels,	Fed antibiotics in 2 separate periods, so could observe effect on withdrawal.

						F. Steers with no antibiotic in feed	particularly to tetracycline, but also to ampicillin. Some evidence of transmission to control animals. Small decline in resistance on withdrawal, and rapid increase again on re-introduction. The prevalence at baselines varied from 20-40%, but increased to ~double with exposure to antibiotics, and fell to about baseline (lower or higher) when antibiotics withdrawn	
Amachawadi 2015 United States	Antibiotic vs none, in presence of normal vs high dose copper.	2 x 2 factorial randomised trial.	Experimental farm / 28 days	Proportion of isolates with resistance.	Gene copy number	A. Feedlot cattle with antibiotic and low-dose copper in feed B. Cattle with high-dose copper and no antibiotic in feed	Resistance to tylosin significantly higher in all 3 treated groups compared with control. Increased copper	No information about resistance levels after ceasing antibiotics.

						C. Cattle with high-dose copper and antibiotic in feed D. Cattle with low-dose copper and no antibiotic in feed	in the diet appears to be associated with increased resistance to some antibiotics	
Beyer 2015 Germany	Antibiotic vs none	2-arm randomised trial.	Experimental farm / 53 days	Proportion of animals with resistance. Also measured stable dust levels.	MIC on agar	A. Pigs treated with antibiotic as IM injection 3 days x 2 occasions. B. Pigs not treated with antibiotic (in the same stable)	Resistant isolates found in all animals by end of experiment (day 53), but appeared earlier in treated animals. Resistant isolates found in air of stables near both groups, suggesting transmission by air from faeces and urine.	
Butaye 2005 Belgium	Antibiotic vs none	3-arm randomised trial. antibiotic until day 42, antibiotic until day 28, no antibiotic.	Experimental farm / 42 days	Mean ratio of resistant to susceptible counts.	MIC on agar	A. Chickens fed antibiotic for 42 days B. Chickens fed antibiotic for 28 days C. Chickens not fed antibiotic	Ratio of resistant to susceptible isolates higher in long-term antibiotic group than shorter-term antibiotic group, in turn higher than control.	No information about resistance levels after ceasing antibiotics.
Chambers 2015 United States	Antibiotic vs none	2-arm randomised trial (n=6).	Experimental farm / 3 days	Proportion of sequences with	Genetic sequencing	A. Cows given antibiotic by injection	Higher ($p<0.10$) resistance to beta-lactams (by a	Small study (6 cows), and only 1 time

				resistance genes.		B. Cows not given antibiotic	factor of ~2). and multidrug resistance (by ~2.5) in antibiotic treated cows.	point immediately post-treatment. No information about resistance levels persisting after ceasing antibiotics.
Checkley 2010 Canada	Antibiotic vs none.	3-arm randomised trial. antibiotic in feed, antibiotic by injection on arrival, no antibiotic.	Experimental feedlot /168 – 248 days	Proportion of animals with resistance.	MIC on agar	A. Cows given antibiotic in feed for 14 days B. Cows given antibiotic by SC injection on first day C. Cows not given antibiotic	Proportion of animals with tetracycline resistance significantly higher in antibiotic -fed animals than injectable and control animals to 100% from 20% by day 15. By Day 210, tetracycline-fed and injected, and control animals converged at about 50-62% resistant.	See Fig 1
Chen 2008 USA	Antibiotic vs none	2-arm randomised	Experimental farm / 21	Number and ratio of	PCR	A. Steers given antibiotics in feed	Resistance to both tetracyline	No information

		trial. antibiotic in feed, no antibiotic.	weeks	resistant genes to susceptible counts.		B. Steers not given antibiotic	and macrolide detected, despite feeding only with tylosin/monensin. Resistance level higher in treated group, but significant levels in control group by 17 weeks.	about resistance levels after ceasing antibiotics.
Coe 2008 United States	Antibiotic vs none	3-arm randomised trial. antibiotic by injection (2 types), no antibiotic.	Experimental feedlot / 210 days	Proportion of isolates with resistance.	PCR	A. Calves given SC injection of antibiotic (tilmicosin) B. Calves given SC injection of antibiotic (florfenicol) C. Calves not given antibiotic	Results not reported by treatment group. Antibiotic resistance was induced in other antibiotic groups High baseline resistance to penicillin and tilmicosin. Rises in index antibiotic resistance was < 1 order magnitude.	Limited results.
da Costa 2010 da Costa 2009 da Costa 2008	Antibiotic vs none.	2-arm randomised trial. antibiotic (3 consecutive types), no antibiotic.	Experimental setting / 33 days	Proportion of isolates with resistance.	Inhibition zone size on agar	A. Chickens given antibiotic (gentamycin, ampicillin) in their drinking water	Higher levels of resistance to tetracycline and erythromycin in medicated group, more pronounced	Pooled (‘ecological’) sampling Data not strong for

Portugal						B. Chickens not given antibiotic	early. Control group resistance levels rise over time. Analysis of farm environment and feed suggests resistance may be introduced through feed, rather than transmission.	hypothesis about transmission of resistance through feed rather than environment.
Davies 1999 United Kingdom	Antibiotic vs none	Compares two groups of pigs, one fed a diet containing antibiotic, the other not fed antibiotics.	Outdoor breeding herd / 12 weeks	Proportion of resistant isolates.	MIC on agar and PCR	A. Piglets fed antibiotic (avilamycin, tylosin) B. Piglets not fed antibiotic	No significant difference between groups in samples taken from slaughtered carcasses	Small sample size. No information about resistance levels after ceasing antibiotics
Delsol 2003 United Kingdom	Antibiotic vs none, in the presence of experimental inoculation with resistance.	3-arm randomised trial. Therapeutic dose of antibiotic (withdrawn after 7 days), sub-therapeutic	Experimental farm / 49 days	Proportion of isolates with resistance.	MIC on agar	A. Pigs fed high-dose antibiotic for 7 days and inoculated with resistant *S. typhimurium* B. Pigs fed low-dose antibiotic for 7 days and inoculated	Similar curves for decline of *S. typhimurium* in all 3 groups, but higher in both antibiotic groups than control. Percentage of tetracycline-resistant *E. coli*	Still high levels of resistance at 6 weeks post-treatment, which is longer than standard withdrawal period pre-

		dose of antibiotic (withdrawn after 7 days), no antibiotic.				C. Pigs inoculated only	higher in high-dose and low-dose groups than control 1-2 weeks after treatment, but not by 3-4 weeks after.	slaughter.
Edrington 2014 United States	Antibiotic vs none	4-arm randomised trial. Distillers' grain feed with no antibiotic, low dose antibiotic, high dose antibiotic, added antibiotic to usual feed.	Experimental setting / 49 days	Proportion of isolates with resistance.	MIC on agar and PCR	A. Cattle with feed not containing antibiotics (negative control)	No difference between feed groups overall in prevalence of vatE, ermB and msrC genes.	No information about resistance levels after ceasing antibiotics.
						B. Cattle with low-dose feed containing distillers' grains where virginiamycin present		
						C. Cattle with high-dose feed		
						D. Cattle fed feed with virginiamycin added (positive control)		
Kaneene 2008 United States	Antibiotic versus withdrawal of antibiotic.	2-arm cluster-randomised trial. Continue antibiotic in feed, withdraw antibiotic from feed.	8 farms / 15 months	Proportion of isolates susceptible.	MIC on agar.	A. Calves changed from antibiotic - containing milk replacer to non-antibiotic milk replacer	Significant reduction in resistant *E. coli* in intervention group (OR for susceptibility 1.3, 95%CI 1.2 to	

						B. Calves continued on antibiotic - containing milk replacer	1.4), but small absolute change in proportion with susceptibility (from 2.0% to 9.3% of isolates susceptible). No statistically significant changes in *salmonella* or *campylobacter*.	
Kanwar 2013, 2014 United States	Antibiotic vs none	2 x 2 factorial trial (N.B. analysed as 4-arm trial). Single animal in pen receives CCFA followed by all receive CTC, all receive CCFA followed by all receive CTC, single animal receives CCFA followed by no antibiotic, all receive	Experimental farm / 26 days	Log$_{10}$ gene copies of resistance genes. Proportion of isolates with resistance.	MIC on agar and PCR	A. Antibiotic by injection followed by antibiotic in feed for all steers in pen B. Antibiotic by injection to 1 steer in pen followed by antibiotic in feed to all steers in pen C. Antibiotic by injection followed by no antibiotic in feed D. Antibiotic by injection to single steer in pen followed by no antibiotic in feed	Trajectory of resistance gene copies similar, with higher levels according to dose of antibiotic.	

		CCFA followed by no antibiotic.				E. Chickens fed therapeutic antibiotic (tylosin) after exposure to inoculated birds.		
Le Devendec 2015 France	Antibiotic vs none	2-arm randomised trial. Antibiotic in water, no antibiotic.	Experimental facility / 33 days (plus manure compost time of 6 weeks)	Ratio of resistant to susceptible genes.	MIC on agar and PCR	A. Chickens fed antibiotic and manure stored B. Chickens fed antibiotic and manure composted C. Chickens not fed antibiotic and manure stored D. Chickens not fed antibiotic and manure composted	Significantly higher levels of resistance genes bla_{TEM} and sul2 in treated group at day 3 (end of treatment period), but not by day 33. No significant differences in resistance in manures.	
McDermott 2005 United States	Antibiotic vs none	2-arm randomised trial. antibiotic in feed, no antibiotic. 4 replicates on original litter. Last replicate no antibiotic in both groups.	Experimental facility / 7 weeks x 4 repeats	Proportion of isolates with resistance.	MIC on agar	A. Chickens fed antibiotic (virginiamycin) B. Chickens not fed antibiotic 4 replicates, keeping same litter throughout. Last replicate no antibiotic in both groups.	Significantly higher resistance levels in treated groups on all 3 replicates. Resistance on litter of treated animals higher than control, but lower than previously treated groups.	
Olumeyan 1986 United States	Antibiotic vs none	2-arm randomised trial –	Setting NR / 8 weeks	Proportion of isolates with resistance.		A. Steers fed antibiotic (salinomycin)	At 4 weeks, significantly higher levels of	Very small study (4 animals)

		antibiotic in feed vs no antibiotic in feed.				B. Steers not fed antibiotic	resistance in treated animals. Higher levels at all time points, but not significantly so.	
Pereira 2014 United States	Antibiotic vs none	2-arm randomised trial. Antibiotic in milk, no antibiotic	Experimental farm / 6 months	Proportion of isolates with resistance.	MIC on agar	A. Calves fed raw milk with low-dose antibiotic s added (similar levels to residues detected in milk withheld from sale due to treatment of animals)	84% of isolates in treated group resistant to 3 or more antibiotic s, compared with 37% in untreated group.	No information about resistance levels after ceasing antibiotics
						B. Calves fed raw milk only		
Platt 2008 United States	Antibiotic vs none	2-arm randomised trial. Antibiotic in feed, no antibiotic.	Experimental feedlot / 33 days	Proportion of isolates with resistance.	MIC on agar	A. Cattle fed antibiotic (chlortetracycline) for 5 days at 3 time points	Higher levels of resistance to tetracycline and ceftiofur in treated group. Some evidence of decline after end of treatment period (15 days post-treatment).	
						B. Cattle not fed antibiotic		
Rollins 1976 United States	Antibiotic versus intermittent antibiotic versus none	6-arm randomised trial: 3 groups antibiotic; 1 group no	Setting NR / 32 weeks	Proportion of isolates with resistance	MIC on agar	A. Pigs fed antibiotic (3 types) until mean 100kg body weight	Results reported in MIC bands, rather than proportion with resistance, except	No information about resistance levels after

		antibiotic but in the same facility as antibiotic groups; 1 group no antibiotic and in a separate facility from antibiotic groups; 1 group intermittent antibiotic in a separate facility.				B. Pigs fed antibiotic until mean 70kg body weight	for intermittent-dose isolated group, where resistance levels matched dose periods.	ceasing antibiotics
						C. Pigs fed antibiotic until mean 40kg body weight		
						D. Pigs not fed antibiotic		
						E. Pigs not fed antibiotic and isolated from all other groups		
						F. Pigs fed antibiotic days 1-14 and 32-43 days and isolated from all other groups		
Scalzo 2004 France	Antibiotic vs none	5-arm randomised trial. Group 1: control, Group 2 monesin 120mg/kg; Group 3: salinomycin 60mg/kg; Group 4: semduramicin 20mg/kg; Group 5: semduramicin 25mg/kg.	New, purposely built animal housing unit / 49 days	No extractable data presented	MIC on agar and PCR	A. Chickens fed antibiotic (monensin) and inoculated with resistant *S. typhimurium*	Resistance phenotype details given, but no comparative analysis between groups.	No information about resistance levels after ceasing antibiotics
						B. Chickens fed antibiotic (salinomycin) and inoculated		
						C. Chickens fed lower dose antibiotic (semduramicin) and inoculated		

						D. Chickens fed higher dose antibiotic (semduramicin) and inoculated		
						E. Chickens inoculated only		
Usui 2014 Japan	Antibiotic vs none	3-arm randomised trial. IM antibiotic, oral antibiotic, no antibiotic.	Experimental facility / 26 days	Resistant CFU ($\log_{10}$) per gram.	MIC on agar	A. Pigs fed antibiotic (norfloxacin) for 5 days	Significantly higher levels of resistance in both treated groups.	Subsequent experiment of housing 5 pigs with sensitive strains (from control group) with a pig with resistance demonstrated the development of resistance in all 5 animals.
						B. Pigs given antibiotic (IM enrofloxacin) by injection for 3 days		
						C. Pigs not given antibiotic		
Wagner 2008 United States	Antibiotic vs none	5-arm randomised trial. Group 1: continuous tylosin; Group 2: continuous CTC; group 3: pulse tylosin; Group 4: pulse CTC; Group 5: no antibiotic (control).	Swine barns / 17 weeks	Proportion of isolates with resistance.	MIC on agar	A. - B. Pigs fed low-dose antibiotic continuously (either chlortetracycline or tylosin)	No significant differences between groups.	Unusually low proportions of resistant isolates compared with other studies in this table.
						C. – D. Pigs fed same antibiotics for 1 week in 4		
						E. Pigs not fed antibiotics		

Other controlled trials and challenge studies

Study ID / Location	Exposure / Comparison	Approach / Design	Setting / Duration	Outcome Measure	Method	Group	Result	Limitations and Comments
Aarestrup 1998 Denmark	Antibiotic vs none.	2-arm controlled trial. Litter divided into antibiotic -fed and no-antibiotic -fed pens in isolation (not clear if randomly allocated)	Experimental farm / 6 weeks	Proportion of isolates with resistance in faeces and skin.	CFU counts on agar.	A. Pigs fed tylosin in feed B. Pigs not fed tylosin	Ratio of fraction resistant between treated and untreated animals rose over 5 days and stayed constant in rectal swab samples (enterococci). Ratio continued to rise steadily over 45 days in skin swab samples (S. hyicus).	No information about resistance levels after ceasing antibiotics.
Alali 2004 United States	Antibiotic vs none, in the presence of inoculation of resistant *E coli*.	2-arm randomised challenge trial. antibiotic -fed or no-antibiotic -fed pens.	Experimental farm / 10 weeks	Proportion of animals with resistance genes detected.	Direct plating	A. Milk replacer containing antibiotic B. Milk replacer without antibiotic	Proportion of antibiotic treated calves with resistant E.coli detected was higher at days 6 and 10 only. No significant difference in duration of shedding of resistance gene.	Laboratory strain of E. coli O157:H7 used, rather than wild-type strain.
Alali 2009b Lowrance 2007 United States	Antibiotic at higher and lower dose, one dose and 3 doses,	2 x 2 factorial controlled trial, with half animals	Experimental farm / 28 days	Gene copy numbers per gram.	Gene copy number / gram	A. High dose antibiotic, 1 dose B. High dose antibiotic, 3 doses	Ratio of resistance gene to ‘housekeeping’	No information about resistance levels after ceasing

	versus none	in each pen not receiving antibiotic. Not clear if randomised.				C. Low dose antibiotic D. Control (no antibiotic). Controls distributed through each of the pens of the treated animals	gene significantly higher in 3-dose high-dose group than control, with a dose-response relationship in other groups. Resistance to ceftiofur most notable in high-dose group.	antibiotics.
Alexander 2010; Beukers 2015 Canada	Antibiotic vs none	2-arm non-randomised trial. antibiotic, no antibiotic.	Experimental setting / 179 days	Proportion of isolates with resistance.	CFUs on agar	A. Beef cattle with antibiotic in feed B. Beef cattle with no antibiotic in feed	Tetracycline and ampicillin resistant isolates higher in treated group in fecal samples, but not in hide samples, carcasses or ground meat. Proportion of resistant isolates decreased after 140 days, and continued to decrease on withdrawal of antibiotics to similar level as control group.	Withdrew antibiotics 28 days prior to slaughter.

Bauer-Garland 2006 United States	Antibiotic vs none, inoculation of resistant strain / inoculation of sensitive strain.	2 x 2 factorial randomised challenge trial. 2 out of 12 chicks in each pen inoculated.	Experimental farm / 7 days	Proportion of animals with resistance (measured in uninoculated animals).	CFUs on agar	A. Chicks inoculated with resistant strain and fed antibiotic in water	Resistant strain detected in 90% of controls sharing a pen with antibiotic treated chicks, compared with 60% of controls sharing a pen with untreated chicks (p=0.03).	
						B. Chicks inoculated with resistant strain and not fed antibiotic		
						C. Chicks inoculated with sensitive strain and fed antibiotic in water		
						D. Chicks inoculated with sensitive strain and not fed antibiotic		
						E. Controls distributed through pens of groups A-D above.		
Benazet 1980 France	Experiment 1: Antibiotic vs none, inoculation of resistant strain / no inoculation. Experiment 2: Antibiotic vs none	Experiment 1: Compares individual chicks inoculated / uninoculated / exposed to antibiotic / unexposed to	Experiment 1: Setting unclear / 33 days Experiment 2: Setting unclear / 48 days	The proportion of isolates with resistance.	Direct plating	A. Chickens fed antibiotic (nosiheptide) and Inoculated with resistant *S. typhimurium*	No significant differences between groups. Experiment was repeated without inoculation with similar results.	No information about resistance levels after ceasing antibiotics
						B. Chickens fed antibiotic but not inoculated		

		antibiotic (4 groups) (challenge trial) Experiment 2: compares chicks exposed vs unexposed to antibiotic				C. Chickens not fed antibiotic, but inoculated only		
						D. Chickens not fed antibiotic, and not inoculated		
Berge 2006 United States	Antibiotic vs none	4-arm controlled trial. Antibiotic in milk replacer + available as therapy, antibiotic for therapy only, no antibiotic but in contact with other pens, no antibiotic in isolation.	Commercial calf farm / 28 days	Proportion of isolates with resistance.	Inhibition zone size on agar	A. Calves fed milk replacer without antibiotics and kept in isolation	Odds ratio for presence of resistant isolates in calves fed antibiotic milk vs no-antibiotic milk was 3.03 (95%CI 1.15 to 7.98)	No information about resistance levels after ceasing antibiotics.
						B. Calves fed milk replacer without antibiotics and kept near other calves		
						C. Calves fed milk replacer without antibiotics, but received antibiotics for clinical disease		
						D. Calves fed milk replacer with tetracycline and neomycin, and received antibiotics for clinical disease		

Bibbal 2007 France	Antibiotic vs none	3 successive series of 6 animals. Series 1 & 2: group 1 – intramuscular ampicillin; group 2 – oral ampicillin; group 3 – control without treatment. Series 3: group 1 & 2 – oral ampicillin; group 3 – control without treatment.	Setting unclear / 7 days	Proportion of isolates with resistance.	No usable data			
Brunton 2014 United	Antibiotic vs none	Alternate assignment to one of two	Dairy farm /13 weeks	Proportion of resistant isolates.	MIC on agar	A. Waste milk with antibiotic residues	Statistically significantly higher levels of	No information about resistance levels after

Kingdom		groups: group fed waste milk containing a variety of antibiotic residues, control group fed powdered milk replacer free from antibiotic.				B. Milk replacer (no antibiotic)	resistance between groups after 3 weeks.	ceasing antibiotics.
Cameron-Veas 2015 Spain	Antibiotic vs none	Compares 4 groups of piglets: untreated control group, group treated with amoxicillin, group treated with ceftiofur, group treated with amoxicillin and ceftiofur.	Conventional commercial pig farm / 73 days	Proportion of animals in each group showing resistance.	MIC on agar and PCR	A. Piglets given antibiotic (ceftiofur) by injection then oral antibiotic (amoxicillin) during preweaning	Higher levels of resistance in treated groups after 1st phase of study, and after 7 days of amoxicillin treatment.	No information about resistance levels after ceasing antibiotics
						B. Piglets given antibiotic by injection only		
						C. Piglets given antibiotic orally during preweaning		
						D. Piglets not given antibiotic		
Cassenego 2011 Brazil	Antibiotic vs none	8-arm randomised trial. Some groups also infected with	Experimental setting / 28 days	Proportion of animals with resistance.	PCR	A. Chickens fed antibiotic (monensin) and infected with *Eimeria* sp.,	High levels of resistance to tetracycline in all groups. No significant	No information about resistance levels after ceasing antibiotics.

		Eimeria sp. No antibiotic and no infection as control. Challenge trial				B.- C. Chickens fed antibiotic (monensin) and infected with *Eimeria* sp., and either probiotic or essential oil	differences in resistance to other antibioticss. Dietary administration (probiotic, essential oil, growth promoter) did not affect resistance.	
						D. - F. Chickens not fed antibiotic, and infected, and given either probiotic, essential oil or growth promoter		
						G. Chickens not fed antibiotic, and infected		
						H. Chickens not fed antibiotic, and not infected		
Cavaco 2008 Denmark	Antibiotic vs none, in presence of resistance by inoculation.	4-arm randomised trial. Antibiotic (3 types), no antibiotic. All animals inoculated with	Experimental setting / 25 days	Counts of resistant coliforms.	CFU/g on agar	A. – C. Three groups of pigs given antibiotics by IM injection for 3 days following inoculation with nalidixic acid resistant *E. coli*	Statistically significantly higher resistance levels to cefotaxime in all 3 antibiotic groups compared with control (approx.	

		nalidixine resistance (challenge trial)				D. Control group inoculated but not given antibiotics	100-fold). Gradual decline in resistance levels by day 25 (well beyond usual withdrawal time prior to slaughter), but still high.	
Daniels 2009 United States	Antibiotic vs none	Experiment 1: 2-arm randomised trial. Antibiotic versus no antibiotic. Experiment 2: herd level association of ceftiofur use with prevalence of ceftiofur resistance [non-randomised]	Experiment 1: Individual rooms / 14 days Experiment 2: 42 dairy herds / June 2006-Sept 2007	Plasmid detection.	Plasmid detection on agar	Experiment: A. Calves given antibiotic by IM injection for 5 days, donor strains of *E. coli*, and recipient strains of *E. coli* and *S. enterica* B. Calves given donor/recipient strains only Observation: 42 herds	Resistance gene detected in 3 of 5 control and 3 of 5 treated calves. First day of detection 3, 7 and 12 in control and 2, 4 and 11 in treated. No difference between groups. In observational study, no association between ceftiofur use and resistance levels was seen.	Experimental study with very small sample size.
DeGeeter 1976 United States	Antibiotic vs none, in presence/ absence of experimental resistance and presence/absence	6-arm randomised trial (partial factorial design). No antibiotic	Experimental farm / 31 days	Sensitivity pattern (zonal diameter).	Direct plating	A. Pigs fed antibiotic and inoculated with nalidixic acid resistant *S. typhimurium*	Resistant bacteria not found in either of the groups kept in isolation (B and E - i.e.	Small study size.

	of isolation.	and no inoculation of resistance and isolated, no antibiotic and no inoculation of resistance but exposed to inoculated animals, no antibiotic and no inoculation of resistance but exposed to antibiotic-treated and inoculated animals, antibiotic and isolated, antibiotic and inoculated, no antibiotic and inoculated.				B. Pigs fed antibiotic and not inoculated, and kept in isolation C. Pigs not fed antibiotic and were inoculated D. Pigs not fed antibiotic and not inoculated, and dispersed into pen with group C. E. Pigs not fed antibiotic and not inoculated, and kept in isolation. F. Pigs not fed antibiotic and not inoculated, and dispersed into pen with group A.	not inoculated, but in the presence and absence of antibiotic), or in group F (no antibiotic, no inoculation, but in contact with those fed antibiotic and inoculated). All animals in both inoculated groups shed resistant bacteria at 2 and 4 days after exposure. Resistant bacteria less likely to be found in unmedicated group over time compared with antibiotic treated group.	
Delsol 2005 United Kingdom	Antibiotic vs none, in the presence of experimental inoculation with resistance.	2-arm randomised trial. Antibiotic, no antibiotic.	Experimental farm / 116 days	MIC geometric mean.	PCR	A. Pigs fed antibiotic for 3 months, following inoculation with resistant *S. typhimurium*	Avilamycin-resistant isolates of enterococci detected only in treated pigs. These isolates were also	Small sample size. No information about resistance levels after ceasing antibiotics.

						B. Pigs inoculated only	resistant to tetracycline.	
Ebner 2000 United States	Antibiotic vs none, in the presence of experimental inoculation with resistance.	4-arm randomised trial. 3 different antibiotic regimens, no antibiotic.	Experimental farm / 70 days	Proportion of isolates with resistance.	Inhibition zone size on agar	A. – C. Pigs fed one of 3 antibiotic regimens, and inoculated with resistant *S. typhimurium*	No difference between antibiotics in resistance stated, but results shown pooled across treatment groups.	No information about resistance levels after ceasing antibiotics.
						D. Pigs not fed antibiotic, but inoculated		
Edrington 2003 United States	Antibiotic vs none, before and after experimental inoculation with resistance.	4-arm randomised trial. 3 different antibiotic regimens, no antibiotic.	Experimental farm / 12 days	Resistant CFU ($\log_{10}$) per gram.	MIC on agar	A. – C. Lambs fed one of three antibiotic regimens, and inoculated with novobiocin and nalidixic acid-resistant *S. typhimurium*	Resistance levels in *E. coli* consistently lower in control group lower after 1st day, but not statistically significantly so. Resistance high in control group.	No information about resistance levels after ceasing antibiotics.
						D. Lambs not fed antibiotic, but inoculated only		
Evangelisti 1975 United States	Antibiotic plus S. Typhimurium inoculation vs no antibiotic and no inoculation	Compares two groups of animals: exposed group (antibiotic in feed and S.	Housed in lots of 10 animals (grouped by species) / 28 days	Proportion of resistant isolates.	MIC on agar	A. Pigs, calves and chickens fed antibiotic (oxytetracycline) and inoculated with susceptible *S. typhimurium*	No significant difference between groups. Some development of resistance in the chickens.	No information about resistance levels after ceasing antibiotics

		Typhimurium inoculation) of swine, calves and chickens, to unexposed group (no antibiotic in feed, no inoculation) of swine, calves and chickens.				B. Animals not fed antibiotic, and inoculated only		
Farnell 2005 United States	Campylobacter jejuni challenge (all animals) plus varying doses of antibiotic (3 groups)	Compares 3 groups of chickens (all challenged by Campylobacter jejuni: no antibiotic in feed (control), 25mg/ml of antibiotic for 3 days, 50mg/ml of antibiotic for 7 days	Chicken research pens / 47 days	Minimum inhibitory concentration (MIC)	MIC on agar	A. Chickens fed higher-dose antibiotic (enrofloxacin) for 7 days, after inoculation with susceptible *C. jejuni*	MIC values significantly higher in treated birds.	Most of paper discussed recovery of *C. jejuni*, rather than resistance.
						B. Chickens fed lower-dose antibiotic for 3 days, after inoculation		
						C. Chickens not fed antibiotic, and inoculated only		
Finlayson 1973 Canada	Antibiotic vs none	Compares 2 groups of piglets: exposed to	Experimental quarters / duration unclear	Proportion of resistant isolates.	MIC on agar	A. Piglets fed antibiotic (chlortetracycline)	Significantly lower numbers of sensitive strains in	No information about resistance levels after ceasing

		antibiotic vs not exposed to antibiotic in feed				B. Piglets not fed antibiotic	antibiotic treated group, with no sensitive strains (100% resistant) on farm with higher dose antibiotic use.	antibiotics
Funk 2006 United States	Antibiotic vs none	Quasi-randomised trial (alternate allocation to treatment). Antibiotic in feed, no antibiotic.	3 swine farms, 22 barns, 2112 pigs / duration not reported	Proportion of isolates with resistance.	MIC on agar	A. Pigs fed with antibiotic (chlortetracycline) B. Pigs not fed antibiotic	Three farms, 1 used preventive and therapeutic antibiotic in both groups. No significant differences in resistance based on counts (small percentage resistant in each group). Odds ratio for an isolate being resistant to tetracycline for antibiotic group compared to no antibiotic group was 7.20 (95%CI 5.95 to 8.71), and for resistance to any antimicrobial 7.67 (95%CI 5.24 to 11.21)	Single time point of measurement (prior to slaughter) only. No information about resistance levels after ceasing antibiotics.

Herrero-Fresno 2016 Denmark	Antibiotic vs none	3-arm controlled trial (not clear if randomised). Antibiotic and inoculation with resistance, no antibiotic with inoculation, no antibiotic and no inoculation.	Experimental setting / 3 weeks	Resistant CFU ($\log_{10}$) per gram.	PCR	A. Pigs fed antibiotic (apramycin) and inoculated with multi-drug resistant *E. coli* B. Pigs not fed antibiotic, and inoculated only C. Pigs not fed antibiotic, and not inoculated	Significantly higher levels of resistance (100-fold) in antibiotic treated group compared with inoculated controls. Levels returned to uninoculated group level by day 8.	
Huang 2014 China	Antibiotic vs none	Compares two groups of pigs, one group fed antibiotic, one group not fed antibiotic (control group).	Commercial pig farm / 61 days	Minimal inhibitory concentration changes.	MIC on agar and PCR	A. Pigs fed antibiotic (ciprofloxacin) for 30 days B. Pigs not fed antibiotic	Significantly higher MIC values in antibiotic group. Levels declined to close to control group by 20 days after withdrawal of antibiotic.	
Inglis 2005 Canada	Antibiotic (various) vs none	Compares 4 groups with varying exposures: chlortetracycline and sulfamethazine; chlortetracyc	Lethbridge Research Centre experimental feedlot / 315 days	Proportion of resistant isolates.	MIC on agar and PCR	A. Calves fed antibiotic (tylosin) B. Calves fed antibiotic (monensin) C. Calves fed antibiotic (virginiamycin)	Significantly lower resistance to ampicillin in virginiamycin, monensin and tylosin groups compared with control, and significantly	No information about resistance levels after ceasing antibiotics

		line alone; virginiamycin, monensin, tylosin phosphate; no antibiotic (control group)				D. Calves fed antibiotic (chlortetracycline)	higher resistance to tetracycline in chlortetracycline group for *C. jejuni* isolates. Significantly higher resistance to erythromycin in chlortetracycline group compared with control, and significantly higher resistance to tetracycline in combination antibiotic group for *C. hyointestinalis* isolates.	
						E. Calves fed antibiotic (chlortetracycline and sulfamethazine)		
						F. Calves fed no antibiotic		
Jiang 2006 United States	Antibiotic vs none	Compares calves exposed to antibiotic to calves unexposed to antibiotic (control group).	Research barn / 17 days	Proportion of resistant isolates	MIC on agar and PCR	A. Calves given antibiotic (ceftiofur) by IM injection for 5 days	Higher MIC values in treated animals, but very small sample size.	No information about resistance levels after ceasing antibiotics
						B. Calves not given antibiotic		
Jimenez-Belenguer 2016 Spain	Antibiotic vs none	Compares 7 groups. 6 groups exposed to varying	Research centre / 49 days	Proportion of resistant isolates	MIC on agar	A. – C. Chickens given antibiotic (amoxicillin) at 3 different doses	High levels of resistance at day 0. Significantly higher levels in all 3 treated	No information about resistance levels after ceasing antibiotics

		doses of amoxicillin; each dose administered to 2 different groups; each group received doses on 3 occasions. Comparison group (7th group) kept unmedicated.				D. Chickens not given antibiotic	groups compared with control.	
Johnson 2015 United States	Antibiotic vs none, in the presence of experimental inoculation with resistance.	4-arm controlled trial (not clear if randomised). Inoculation followed by high dose antibiotic, inoculation followed by low dose antibiotic, inoculation alone, no inoculation and no antibiotic.	Experimental setting / 35 days	Proportion of isolates with resistance.	PCR	A. Pigs fed high-dose antibiotic (chlortetracycline) and inoculated with resistant *E. coli* B. Pigs fed low-dose antibiotic and inoculated C. Pigs not fed antibiotic, and inoculated only D. Pigs not fed antibiotic and not inoculated	High dose antibiotic group had higher levels of resistant *E. coli* at all time points after day 3. Low dose not significantly different from inoculated control. Uninoculated control negative for resistance at all time points.	No information about resistance levels after ceasing antibiotics.
Kempf 2013 France	Antibiotic vs none	Compares turkeys fed antibiotic in	Turkey farms with flocks sized from	Proportion of resistant isolates	MIC on agar and PCR	A. Turkeys given antibiotic (paromomycin)	Significantly higher level of resistance to	No information about resistance levels after

		feed, to turkeys that were not fed antibiotic in feed.	3300-11,500 birds / 180 days			B. Turkeys not given antibiotic	amoxicillin in treated group at day 90 and to streptomycin at day 60.	ceasing antibiotics
Khachatryan 2004 United States	Antibiotic vs none	Compares calves that received diets supplemented with antibiotic to calves that received diets without antibiotic.	Dairy farm at Washington State University / 12 weeks	Proportion of resistant isolates	MIC on agar and PCR	A. Calves fed antibiotic (oxytetracycline) B. Calves not fed antibiotic	Complex data for patterns of resistance given.	No information about resistance levels after ceasing antibiotics
Khachatryan 2006 United States	Antibiotic vs none	3-arm controlled trial (sequential allocation). Dietary supplement + antibiotic, dietary supplement alone, no supplement	Experimental farm / 3 months	Proportion of isolates with resistance.	MIC on agar	A. Calves given antibiotic in feed supplement B. Calves given same feed supplement with no antibiotic C. Calves not given feed supplement	Both supplement-fed groups had higher levels of resistance than controls	Not clear if higher rate in supplement but no antibiotic group is due to transmission from antibiotic group animals, or effect of supplement in selecting for resistant strains.
Kim 2005 United States	Antibiotic vs none	2-arm controlled trial (not clear if randomised).	Experimental farm / 75 days	Proportion of isolates with resistance.	MIC on agar	A. Piglets fed several antibiotics during growth phase	Significantly higher level of resistance to apramycin in treated group	

		Antibiotic regimen (3 antibiotics), no antibiotic in feed.				B. Piglets not fed antibiotic	after feeding with apramycin, but reduced to control group level over time. Significantly higher level of resistance to tetracycline in treated group after feeding with tetracycline. Level continued to rise.	
Kobe 1996 Germany	Antibiotic vs none	Compares broiler chickens that received diets supplemented with antibiotic (furazolidone) to broiler chickens that received diets without antibiotic.	Setting / Duration unclear (possibly reported in text of the full article, which is available in German only)	MIC values	MIC on agar	A. Chickens fed antibiotic (furzolidone) B. Chickens not fed antibiotic	Statistically significant higher MIC values in treated birds.	No information about resistance levels after ceasing antibiotics
Kobe 1995 Germany	Antibiotic vs none	Compares broiler chickens that received	Setting / Duration unclear (possibly	MIC values	MIC on agar	A. Chickens fed antibiotic (oxytetracycline)	Statistically significant higher MIC values in treated	No information about resistance levels after ceasing

		diets supplemented with antibiotic (oxytetracycline) to broiler chickens that received diets without antibiotic.	reported in text of the full article, which is available in German only)			B. Chickens not fed antibiotic	birds.	antibiotics
Ladely 2007 United States	Antibiotic vs lower dose antibiotic	3 replicated experiments. In all: all chickens were exposed to macrolide-susceptible C.jejuni or C.coli. At 2 weeks of age, chickens were either exposed to subtherapeutic or therapeutic concentration of an antibiotic.	Chicken farm / 6 weeks	Proportion of resistant isolates	MIC on agar and PCR	A. Chickens fed subtherapeutic antibiotic (tylosin) after contact with birds inoculated with susceptible *C.jejuni* or *C.coli*.	More resistance detected in lower-dose antibiotic group than higher dose in both *C.jejuni* and *C.coli*	Most of paper is about recovery of Campobacter, rather than resistance.
						B. Chickens fed therapeutic antibiotic (tylosin) after exposure to inoculated birds.		

Lin 2007 United States	Antibiotic vs none	Several in vivo and in vitro experiments. Exp 1: chickens inoculated with C.jejuni 700819 and S3B; 2 groups	Setting unclear / 20 days	Log$_{10}$ CFU/g feces	MIC on agar and PCR	A. – C. Chickens fed antibiotic (tylosin) for 3 days, after inoculation with susceptible C.jejuni or C.coli. One group fed tylosin 3 times.	No erythromycin resistance detected in untreated chickens, but detected in medicated chickens on days 31 and 38 on 1st	No information about resistance levels after ceasing antibiotics

		received a single treatment with tylosin; 2 groups served as non-treated controls. Exp 2: 2 groups of chickens inoculated with a mix of C.coli strains AW-II-35 and AW-II-37; one group received a single tylosin treatment; the other group served as control. Exp 3: Chickens were infected with C.jejuni 700819; one group received 3 tylosin treatments at weekly intervals; one group was non-treated control.				D. – F. Chickens not fed antibiotic after inoculation 3 repetitions of the experiment	experiment. Significantly lower resistance in untreated group in 2nd experiment also.	

Logue 2010 United States	Antibiotic vs none	Antibiotic was applied to one half of a c. 30,000 turkey flock; the other half served as control (no antibiotic application).	Large-scale turkey production facility / March-July 2007	Prevalence of resistance in animals.	MIC on agar and PCR	A. Turkeys fed antibiotic (tylosin) in 3 periods of 3 days	Significantly higher resistance to erythromycin in treated group. Only 4 isolates resistant in untreated group.	No information about resistance levels after ceasing antibiotics
						B. Turkeys not fed antibiotic		
						B. Steers given antibiotic as SC injection of reduced dose		
						C. Steers given antibiotic as SC injection x 3 doses		
						D. Steers not given antibiotic		
Marosevic 2014 Czech Republic	Antibiotic vs none	Compares 4 groups, all challenged with E faecalis harbouring plasmid pAMb1 (carrying the erm(B) gene). Groups 1, 2, 3 were given different antibiotics in	Setting unclear / 42 days	Number of resistant isolates.	MIC on agar and PCR	A. Chickens fed antibiotic (tylosin) after inoculation with plasmid carrying erm(B) gene	Significant within-group changes in resistance levels, however between-group results not given.	No between-group results available.
						B. Chickens fed antibiotic (lincomycin) after inoculation		
						C. Chickens fed antibiotic (chlortetracycline) after inoculation		

		feed; group 4 was not given antibiotics (control group)				D. Chickens inoculated only		
Molitoris 1986 United States	Antibiotic vs none	Compares chickens fed rations supplemented with chlortetracycline, to chickens fed antibiotic-free diet.	Chick hatcheries / 1979-1981	Percentage of resistant strains.	MIC on agar	A. Chickens fed antibiotic (chlortetracycline) for 42 days B. Chickens not fed antibiotic	Mixed results. No clear pattern of favouring either treated or untreated group in resistance levels.	No information about resistance levels after ceasing antibiotics
Moodley 2011 Denmark	Antibiotic vs none	Compares four variously treated groups: tetracycline and zinc; zinc alone; tetracycline alone; non-treated (control) group. Each	Isolation facilities at the University of Copenhagen / 21 days	Mean MRSA counts (CFU/sampling) from nasal swabs.	MIC on agar and PCR	A. Pigs fed antibiotic (tetracycline) for 7 days with zinc B. Pigs fed antibiotic without zinc C. Pigs fed zinc without antibiotic D. Pigs not fed antibiotic or zinc.	Significantly higher MRSA in nasal cavity of pigs treated with combination of antibiotic and zinc and for each treatment separately, compared with control. No significant interaction	

		group contained two MRSA-positive and two MRSA-negative pigs.				Each group contained both MRSA positive and negative animals	detected.	
Nivas 1976 United States	Antibiotic vs none	Experiment 1: Group 1 was not fed antibiotic in feed (control); groups 2, 3, 4, 5 were fed different doses of antibiotic in feed. All poults in all groups were orally given S typhimurium phage type T45 Experiment 2: as above,	Setting NR / 16 days (exp 1) and 18 days (exp 2)	No extractable data was presented.		Experiment 1: A. – D. Turkeys fed antibiotic (chlortetracycline) in four different doses	No comparative results given.	Small numbers of isolates detected (older study – 1976).
						E. Turkeys not fed antibiotic		
						Experiment 2 A. – D. As for 1, but inoculated with resistant *S. typhimurium*		
						E. Turkeys not fed antibiotic, but inoculated only		
						F. Turkeys not fed antibiotic and not inoculated		

		except groups 2-5 also infected with S typhimurium organisms at higher concentration				Experiment 1: A. – D. Turkeys fed antibiotic (chlortetracycline) in four different doses		
Sharma 2008 Wu 2011 (subset with genetic testing) Canada	Antibiotic vs none	3-arm non-randomised, controlled trial. Single antibiotic, two antibiotics, no antibiotic.	Experimental facility / 197 days	Proportion of isolates with resistance.	MIC on agar and PCR	A. Calves fed antibiotic (chlortetracycline)	Significantly higher resistance levels in both treated groups. Increasingly similar genotypes by day 197 suggests animal-to-animal strain transmission, but data not definitive.	No information about resistance levels after ceasing antibiotics
						B. Calves fed 2 antibiotics (chlortetracycline and sulfamethozine)		
						C. Calves not fed antibiotic		
Stapleton 2010 United Kingdom	Antibiotic versus lower dose antibiotic	Exp 1: chickens were dosed with C jejuni 81116P, then treated with 3 different doses of enrofloxacin (50, 125, 250 ppm) for 5, 3, 1 days respectively.	Research facility / 35 days	Geometric mean CFU per gram caecal contents	MIC on agar	Experiment 1: A. Chickens fed antibiotic (enrofloxacin) low dose for 5 days, and inoculated with *C.jejuni*	Experiment 1: After 48 hours, 68%, 76% and 71% of isolates resistant to ciprofloxacin, and ≥ 93% after 72 hours. Experiment 2: At 48 hours, proportion of resistant isolates was 34%, 50%,	No information about resistance levels after ceasing antibiotics
						B. Chickens fed antibiotic moderate dose for 3 days, and inoculated		

		Exp 2: chickens were dosed with C jejuni 81116P, then separated into 6 groups (12, 25, 50, 125, 250, 500ppm enrofloxacin); each group treated for 3 days.				C. Chickens fed antibiotic high dose for 1 day, and inoculated Experiment 2: A. – F. As for experiment 1, but with 6 doses of enrofloxacin given for 3 days	50%, 99%, 100% in the dose groups, and 82% in lowest dose group, 100% in all other groups after 72 hours.	
Takahashi 2005 Japan	Antibiotic vs none	Exp 1: C jejuni ATCC 33560^T added to drinking water at 17 days old for all chickens.	Research facility / 28 days	Proportion of resistant isolates	MIC on agar, and PCR	A. Chickens fed antibiotic (enrofloxacin) for 3 days, and inoculated with sensitive *C.jejuni*	Experiment repeated. No resistant isolates found in control group in either experiment.	No information about resistance levels after ceasing antibiotics

		One group of chickens received 50ppm enrofloxacin for 3 days (at 24 days old); the other group served as control. Exp 2: C jejuni ATCC 33560^T added to drinking water twice (at 18 & 23 days old) for all chickens. One group received 50ppm enrofloxacin for 3 days (at 32 days old); the other group served as control.				B. Chickens not fed antibiotic, but inoculated only	Contradictory results in the repeats in the experimental group. High levels of resistance to nalidixic acid, enrofloxacin and olfoxacin in 2nd experiment.	

van der Horst 2013 The Netherlands	Varying levels and types of antibiotics vs none	Tested 3 antibiotics (amoxicillin, enrolfoxacin, oxytetracycline). Each antibiotic population was split into 4 different levels of exposure (therapeutic dose, 75% of the therapeutic dose, 2.5% of the therapeutic dose, control group).	chickens were housed in HEPA-filtered isolators / 16 days	Proportion of resistant isolates	MIC on agar	A. – C. Chickens fed antibiotic (amoxicillin) in 3 doses (therapeutic, 75% of therapeutic, 2.5% of therapeutic	Dose-response relationship seen in resistance to oxytetracycline and aimoxicillin. Levels decline after withdrawal of antibiotic, but still 20-40% in 100% and 75% groups at 16 days. Resistance to enrofloxacin lower than the other 2 antibiotics. Oxytetracycline induced resistance to amoxicillin (close to 100% at 2 days in higher dose groups) and amoxicillin induced resistance to oxytetracycline (80% in high dose group)	Not clear if antibiotics are selecting for strains already resistant, or inducing gene transfer, as PCR characterisation of genes was not done.
						D. – F. Chickens fed antibiotic (oxytetracycline) in 3 doses (therapeutic, 75% of therapeutic, 2.5% of therapeutic		
						G. – I. Chickens fed antibiotic (enrofloxacin) in 3 doses (therapeutic, 75% of therapeutic, 2.5% of therapeutic		
						J. Chickens not fed antibiotics		
Wierup 1975 Sweden	Antibiotic vs none	Compares 4 groups: zinc bacitracin;	Setting unknown; duration	Incidence of antibiotic resistance	MIC on agar	A. Calves fed antibiotic (bacitracin)	Combined group had higher resistance than	

		zinc bacitracin & lactosat; oxytetracycline and zinc bacitracin; lactosat only (no antibiotic exposure)	unknown. (Possibly provided in the article, which is in Swedish)			B. Calves fed antibiotic (oxytetracycline + bacitracin then bacitracin only) C. Calves not fed antibiotic, then antibiotic (bacitracin) D. Calves not fed antibiotic	control. No significant difference in the other 3 groups. Increase in resistance to other antibiotics observed in all groups, including control.	
Zaheer 2013 Canada	Antibiotic vs none	Compares 4 groups: tilimicosin (single injection); tulathromycin (single injection); tylosin in feed for 28 days; control group (no antibiotic).	Research Centre / 28 days	Proportion of resistant isolates	MIC on agar and PCR	A. Cattle given antibiotic (tilmicosin) by SC injection day 1 B. Cattle given antibiotic (tulathromycin) by SC injection day 1 C. Cattle fed antibiotic (tylosin) for 28 days D. Cattle not given antibiotic	Highest levels of resistance to erythromycin seen in tylosin-fed animals at day 7, but no statistically significant differences between antibiotic groups. All 3 antibiotic groups statistically significantly higher than control out to end of experiment at 28 days.	

Cohort studies								
Study ID / Location	**Exposure / Comparison**	**Approach**	**Setting / Duration**	**Outcome Measure**	**Method**	**Group**	**Result**	**Limitations and Comments**
Akwar 2008 Canada	Antibiotic vs none	Compares farms using antibiotic in feed, and farms that do not.	Farrow-to-finisher pig farms / 7 months (Ontario farms) or 4 months (BC farms)	Proportion of isolates with resistance.	MIC on agar	A. Pigs on farms using antibiotic in feed B. Pigs on farms not using antibiotic in feed	80% of *E. coli* isolates from antibiotic farms had resistance to 2 or more antibiotics, compared with 52% from farms not using antibiotics ($p<0.01$)	No information about resistance levels after ceasing antibiotics
Alali 2010b United States	Antibiotic vs none	Compares organic farms (free of antibiotic agents) and conventional farms (use antibiotic in feed)	broiler farms / 10 weeks for two flocks	Proportion of isolates with resistance.	MIC on agar and PCR	A. Chickens on conventional farms B. Chickens on organic farms	Fecal shedding of *Salmonella* lower in organic farm. *Salmonella* isolates from conventional farms had significantly more resistance to cefoxitin, ceftiofur, streptomycin, sufisoxazole and tetracycline.	No information about resistance levels after ceasing antibiotics
Andersen 2015 Denmark	Antibiotic vs none	Compares herds with no antibiotic use, to herds	Conventional medium to large pig herds / 7 months	Probability of isolating resistant isolates	PCR (ESC-R genes)	A. Pigs on farms where cephalosporins-III/IV used	Risk ratio for resistance in *E.coli* in exposed farms	No information about resistance levels after ceasing

		with antibiotic use				B. Pigs on farms where cephalosporins-III/IV not used	was 4.74 (95% CI 2.0 to 11.5).	antibiotics
Baron 2014 France	Antibiotic vs none	30 broiler flocks (15 treated, 15 non-treated); 22 flocks of future laying hens (12 treated, 10 non-treated)	free range broiler farms / 77 days; future laying hens farms / 200 days	Proportion of isolates with resistance	MIC on agar, and PCR	A. Chickens from farms where antibiotic (ceftiofur) given *in ovo*	Percentage of *E.coli* isolates with resistance in exposed groups was 35% and 46%, compared with 11% and 22% in unexposed groups.	No information about resistance levels after ceasing antibiotics
						B. Chickens from farms where antibiotic not given		
						C. Chickens from farms where antibiotic (ceftiofur) given SC at 1 day of age		
						D. Chickens from farms where antibiotic not given		
Benedict 2015 Canada	Association between antibiotic exposure and prevalence of resistance (regression)	Using regression techniques, analysed associations between antimicrobial exposures and antimicrobial resistance in 305 cattle	4 commercial beef feedlots employing production practices typical for large feedlots in the US and Canada / Sept 2007-Jan 2010	Proportion of isolates with resistance.	MIC on agar	A. Cattle given antibiotics as part of routine care	Odds ratio for resistance to tetracycline for animals given/not given parenteral or in-feed tetracycline were statistically significantly different from 1 (1.32 for parenteral, 1.18	No information about resistance levels after ceasing antibiotics

							for in-feed). Odds ratio for resistance to trimethoprim-sulfamethoxazole for animals given/not given parenteral tetracycline was also significant, at 2.59 (95% CI 1.72 to 3.89).	
Callens 2015 Belgium	Antibiotic vs none	Compares the antimicrobial exposure and prevalence of resistance amongst healthy animals.	Farrow-to-finish pig farms / 3 months	Odds ratios (risk of antimicrobial exposure for prevalence of resistant isolates).	MIC on agar	A. Sows and piglets given antibiotics as part of routine care B. Sows and piglets not give antibiotics	Piglets from sows treated with lincomycin-spectinomycin had higher levels of resistance to ampicillin (OR 8.12), enrofloxacin (OR 7.50), streptomycin (OR 56.98), and trimethoprim-sulfadiazine (OR 16.17), suggesting transfer of resistant bacteria, or selection of resistant	No information about resistance levels after ceasing antibiotics

							population.	
Duse 2015 Sweden	Antibiotic vs none	Examines risk factors for increased prevalence of resistant isolates, including exposure vs. no exposure to antibiotics on 243 dairy farms.	Dairy farms / Oct 2011-Sept 2012	Proportion of resistant isolates.	MIC on agar	A. Calves given colostrum, transition milk or waste milk from cows treated with antibiotics	No significant effect of feeding waste colostrum or transition milk. Significantly higher resistance to streptomycin and nalidixic acid in calves fed waste milk (OR 1.62 and 4.34 respectively).	No information about resistance levels after ceasing antibiotics
						B. Calves not given colostrum or milk from cows treated with antibiotics		
Heuer 2002a Denmark	Antibiotic stopped vs never exposed	Compares 162 broiler flocks with different histories of	Boiler farms using conventional or organic rearing / Oct	Proportion of resistant isolates.	MIC on agar, and PCR	A. Chickens from farms where antibiotic (avoparcin) previously used	VRE detected in 74.3% flocks from avoparcin-using farms, compared with	Avoparcin not used for 5 years prior to this study.

		exposure to antibiotic (conventional farms vs organic farms), 5 years after the antibiotic was banned.	1998- Feb 2001			B. Chickens from farms where antibiotic never used	9.1% from farms where avoparcin never used.	

Juntunen 2010; Finland	Antibiotic vs none vs withdrawal of antibiotic	Compares treated sows and piglets; pigs treated with an antibiotic (both during treatment and after withdrawal); pigs after antibiotic had not been administered on the farm for 7 months.	Large pig farm / October 2007 to February 2008	Proportion of resistant isolates	MIC on agar, and PCR	A. Pigs fed tylosin B. Pigs not fed tylosin C. Pigs not fed tylosin, 7 months after tylosin use ceased.	19.6% of isolates resistant to ciprofloxacin, 46.4% resistant to erythromycin, 19.6% resistant to nalidixic acid and 25.0% resistant to streptomycin in antibiotic group, compared with 0 – 5% in untreated group. At 7 months, 0 – 9.7% (erythromycin) of isolates with resistance.	
Keelara 2013; Quintana-Hayashi 2012 United States	Antibiotic vs none	Compares pigs raised on 10 conventional farms and pigs raised on 8 antimicrobial-free farms (35 pigs per farm)	Conventional and antimicrobial-free farms / Oct 2008-Dec 2010	Proportion of resistant isolates	MIC on agar	A. Pigs on conventional farms (antibiotics used for growth, prophylaxis and treatment) B. Pigs on antibiotic-free farms (animals with disease removed prior to giving antibiotics)	80% of *Salmonella spp* isolates resistant to tetracycline in conventional farm samples compared with 27% in antibiotic-free samples. 83% of Campylobater spp isolates resistant to tetracycline in conventional	No information about resistance levels after ceasing antibiotics

							farm samples compared with 49% in antibiotic-free samples.	
Mathew 1999 United States	Higher dose antibiotic vs lower dose antibiotic	Compares 3 low antibiotic use swine farms and 7 high antibiotic use swine farms 10 swine farms; swine farms ranged in size from 1500-10,000	Swine farms / 63 days	Proportion of resistant isolates	MIC on agar	A. Pigs from high antibiotic use farms (feed-based or injectable) B. Pigs from low antibiotic use farms (subtherapeutic tetracyclines for brief periods)	Generally lower resistance in low-use farms at all pig ages. Multiple resistance highest at 35 – 63 days of age on high-use farms, most commonly to neomycin, gentamicin and oxytetracycline.	No information about resistance levels after ceasing antibiotics
Morley 2011 United States	Antibiotic vs none	Compares 40 pens (4557 animals) of feedlot cattle that were not exposed to antimicrobial drugs, to 44 pens of cattle (4913 animals) that were exposed to	3 large commercial feedlots / 27 weeks	Proportion of resistant isolates	MIC on agar	A. Cattle fed antibiotic B. Cattle not fed antibiotic	No significant difference in resistance between the groups, although slightly higher levels of resistance to sulfamethoxazole and tetracycline in antibiotic-fed group.	No information about resistance levels after ceasing antibiotics

		antimicrobial drugs						
Nulsen 2008 New Zealand	Antibiotic vs none	Compares 3 conventional farms (with exposures to various antibiotics) to an organic farm (no exposure to antibiotic).	Conventional and organic pig farms / March to Oct 2001	Proportion of resistant isolates	MIC on agar	A. Pigs from conventional farms (antibiotics in feed, and by injection) B. Pigs from an organic farm	Significantly higher resistance to streptomycin and tetracycline on conventional farms in *E.coli*, and to erythromycin, streptomycin, tetracycline, and virginiamycin in *Enterococcus* spp. 2 – 6% of isolates resistant on organic farm (neomycin, streptomycin, tetracycline, erythromycin, tetracycline).	No information about resistance levels after ceasing antibiotics
Petersen 2002 Thailand	Antibiotic vs none	Compared 8 integrated fish farms (4 chicken-fish farm; 2 duck-fish; 2 pig-fish); to 4 control fish farms (no	Integrated fish farms and non-integrated fish farms / each sampled either Oct 1999-Jan 2000 or Apr 2000-June 2000	Proportion of resistant isolates	MIC on agar	A. Integrated broiler chicken-fish farms with antibiotic use B. Integrated layer chicken-fish farms with antibiotic use C. Integrated	Resistance in *Acinetobacter* spp from water sediment higher for ciprofloxacin (33%) at broiler-fish farm compared with 2.6% at control	No information about resistance levels after ceasing antibiotics

		deliberate input of animal waste or antimicrobials)				duck-fish farms with antibiotic use D. Integrated pig-fish farms with antibiotic use E. Control farms with no use of manure in fish ponds and no use of antibiotics	farms. Resistance to oxytetracycline higher (66%) at pig-fish farms than control (20%). Higher resistance to chloramphenicol higher at control farms.	
Rajala-Schultz 2009 United States	Antibiotic vs lower dose antibiotic	Compares two commercial (high risk of mastitis) and two institutional (low risk of mastitis/uninfected) dairy farms before and after antimicrobial dry cow therapy	Herds NR	Proportion of resistant isolates	MIC on agar, and PCR	A. High-risk cows treated with intramammary antibiotic B. Low-risk cows treated with intramammary antibiotic C. Low-risk cows not treated with antibiotic Groups B & C randomly allocated	Isolates from high-risk cows had OR 2.3, 2.8, 2.4 of being resistant to oxacillin, penicillin-novobiocin and sulfadimethoxine compared with untreated low-risk cows.	No information about resistance levels after ceasing antibiotics
Sato 2004 Japan	Antibiotic vs none	Compares organic dairy herds (antibiotics rarely used) to	Conventional and organic farms / 7 months (March-September)	MIC distributions	MIC on agar	A. Dairy cows on organic farms where antibiotics rarely used for calves and never for	Did not find significant difference of MIC distributions for tetracycline	No information about resistance levels after ceasing antibiotics

		conventional herds (antibiotics routinely used)				cows	between the two types of farm.	
						B. Dairy cows on conventional farms, where antibiotics routinely used for animals of all ages		
Scott 2012 Canada	Antibiotic vs none	Models (logistic regression) risk factors (including antimicrobial use vs. no use) for antibiotic resistance	49 sheep producers / 1-year period between 2006 and 2008.	Odds ratios	MIC on agar	A. Sheep given or not given each antibiotic (penicillin, tetracycline in feed, tetracycline injection, tilmicosin, sulphonamide, trimethoprim-sulfonamide)	No resistance detected in *Salmonella* spp isolates. OR for resistance was 2.6 for trimethoprim-sulfonamide and 4.8 for tetracycline between sheep where injectable sulphonamide used compared with controls, in *E.coli* isolates.	No information about resistance levels after ceasing antibiotics

Interrupted time series								
Study ID / Location	**Exposure / Comparison**	**Approach**	**Setting / Duration**	**Outcome Measure**	**Method**	**Group**	**Result**	**Limitations and Comments**
Hiki 2015 Japan	Percentage of resistant isolates before and after withdrawal of off-label ceftiofur use in hatcheries	Interrupted Time Series	Chicken hatcheries in Japan / 2000-2013	Percentage of resistant isolates (before and after withdrawal of off-label ceftiofur use in hatcheries)	MIC on agar	Monitoring study on 362 farms, before and after ban on ceftiofur	Percentage of isolates with resistance to cephalosporin rose from 0-5% to 17-22% with the use of ceftiofur, then dropped after the ban back to 5% after 2 years.	
Smith 1975 United Kingdom	Percentage of resistant isolates of E.coli before and after ban of feeding tetracyclines to pigs for growth promotion	Interrupted Time Series	Pigs brought to a Chelmsford Market (UK) / 1956-72	Percentage of resistant isolates (before and after ban on tetracyclines for growth promotion in pigs)	Not reported	Monitoring study before and after ban on tetracyclines	Percentage of samples with all E.coli resistant to tetracycline rose from 18% to 64% during the use of tetracycline, and fell to 23-41% 2-5 years after the ban. No change in resistance to other antibioticss	Only 1 or 2 measurements prior to ban

Summary of results for PICO question 1

The heterogeneity between food animals, class of antimicrobial, micro-organisms displaying resistance, and animal housing, together with the multiplicity of research methods, preclude any quantitative summation.

Does limiting or stopping of antimicrobials administration to food animals result in a decrease in antimicrobial resistance?

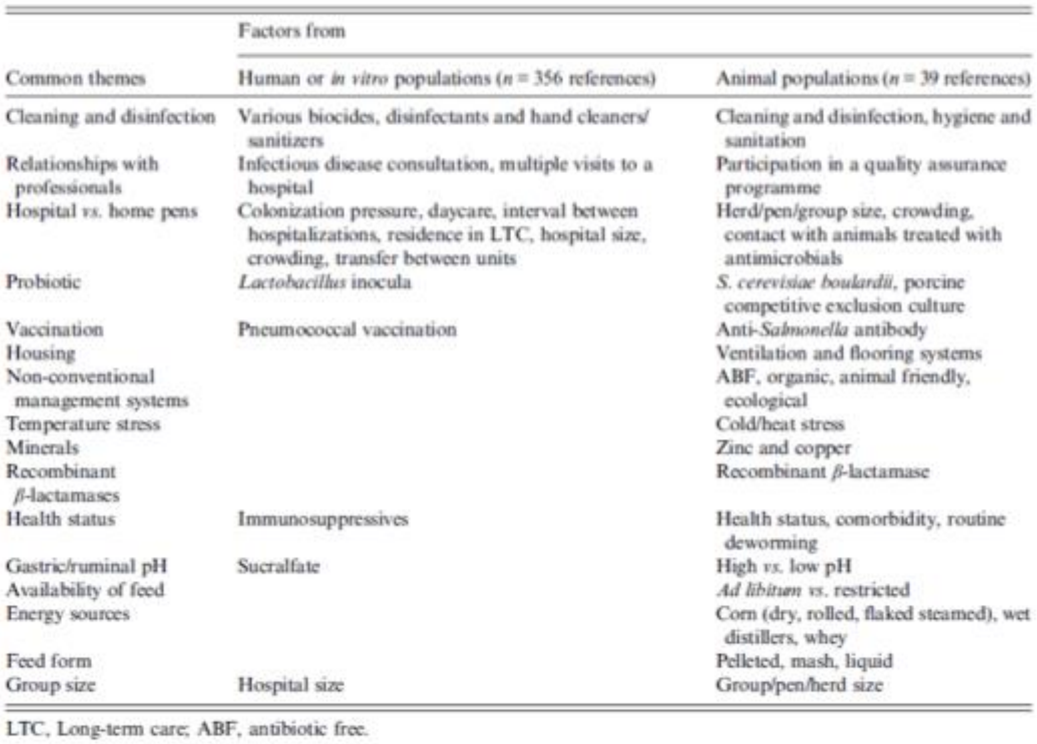
The primary evidence to test this question is good, despite being reported in widely disparate ways, (precluding meta-analysis). Several studies had a design method category sufficiently free from risk of bias (‘quality’), support the hypothesis that limiting antimicrobial use results in less resistance (mostly in animal faeces) among food animals (Delsol 20013; Le Devendec 2015; McDermott 2005; Platt 2008).

The fall in resistance is an exponential decay to a baseline after some weeks. The quantity of the decrease is often modest, for example in Kaneene 2008, stopping tetracycline administered to calves in their milk resulted in an increase in *E coli* susceptibility from a baseline of 2% to ~17% in the first 3 months, which dropped back slowly with time to 5% (with a control baseline of 2-4%).

There were some exceptions. For example the decay was similar for those provided high dose chlortetracycline, low does or none, after inoculation with resistant *Salmonella typhimurium*, in Delsol 2013. Nor was there evidence of an increase in antibiotic resistance on the carcasses of slaughtered animals (for example, pigs fed antibiotic supplemented feed compared to those not in Davies 1999).

Other observations from the literature review for PICO question 1

1. As expected, administering antibiotics to animals causes increased antibiotic resistance in many studies, (eg Eber 2000; Pereira 2014). This may occur even if the concentration of antibiotic is tiny, such as at the level to mimic common practice of use of food unfit for humans because of the ‘residue’ of antibiotic in un-supplemental feeds, in Pereira 2014.
2. Use of one antibiotic in food animals often resulted in the development of resistance to a different antibiotic (eg, Chen 2008; Coe 2008; Platt 2008)
3. Unexpectedly, there was often a high baseline proportion of antibiotic resistance in food animals. (eg Alexander 2015/4; Checkley 2010; Coe 2008; Kaneene 2008), before administration of antibiotic.
4. In line with this, control animals often developed antibiotic resistance over time with no direct exposure when in adjoining environment(da Costa 2009), (Beyer 2015), or animals not directly given antibiotics (Kanwar 2014)
5. The increase in resistance from starting antibiotics was often small (less one order greater than baseline) (Alexander 2015/4; Checkley 2010; Coe 2008)
6. Alternative interventions were encountered among some of these papers:
    a. A copper enriched diet may increase resistance prevalence (Amachawadi 2015);
    b. Composting chicken manure reduced the resistance plasmid only slightly (Le Devendec 2015)
    c. A scoping review of non-antimicrobial interventions for mitigating antimicrobial resistance found interventions that could be categorised into 16 common themes (Fig R6, below) The categories for humans were unsurprising: cleaning and disinfection; relationship with professionals; hospital *vs* home; probiotics; vaccination; health status; gastric pH; group (hospital) size.
    But there were similar categories for animals: cleaning and disinfection; relationship with professionals; hospital vs home pens; probiotics; vaccination; housing; non-conventional management systems; temperature stress; minerals; recombinant beta-lactamases; health status; ruminal pH; energy sources; feed form; group size.[12]



	Factors from	
Common themes	Human or *in vitro* populations (*n* = 356 references)	Animal populations (*n* = 39 references)
Cleaning and disinfection	Various biocides, disinfectants and hand cleaners/ sanitizers	Cleaning and disinfection, hygiene and sanitation
Relationships with professionals	Infectious disease consultation, multiple visits to a hospital	Participation in a quality assurance programme
Hospital *vs.* home pens	Colonization pressure, daycare, interval between hospitalizations, residence in LTC, hospital size, crowding, transfer between units	Herd/pen/group size, crowding, contact with animals treated with antimicrobials
Probiotic	*Lactobacillus* inocula	*S. cerevisiae boulardii*, porcine competitive exclusion culture
Vaccination	Pneumococcal vaccination	Anti-*Salmonella* antibody
Housing		Ventilation and flooring systems
Non-conventional management systems		ABF, organic, animal friendly, ecological
Temperature stress		Cold/heat stress
Minerals		Zinc and copper
Recombinant *β*-lactamases		Recombinant *β*-lactamase
Health status	Immunosuppressives	Health status, comorbidity, routine deworming
Gastric/ruminal pH	Sucralfate	High *vs.* low pH
Availability of feed		*Ad libitum vs.* restricted
Energy sources		Corn (dry, rolled, flaked steamed), wet distillers, whey
Feed form		Pelleted, mash, liquid
Group size	Hospital size	Group/pen/herd size

LTC, Long-term care; ABF, antibiotic free.

Figure R6 Non antimicrobial approaches to reducing resistance (from Murphy 2016 Table 5[12])

However the review concluded that at present, no modifiable antimicrobial factors/interventions can be recommended for adoption until further research quantifies the benefits.

Discussion

a) Systematic review vs. rapid systematic reviews

Because the present review was conducted to a 4½ month timeline (from 16 May to 30 September 2016), several rapid systematic review methods were adopted. Existing comparisons of rapid systematic reviews and full systematic reviews have found their overall conclusions are largely concordant.[8 10] Nevertheless, where rapid systematic review methods were adopted, they are explicitly identified.

A strength of our method is that it took a systematic approach to the search strategy, which is reproducible and objective (with *a priori* methods established), so that, although doubtless there will be some studies that were missed, the sample of the literature found and analysed will be largely representative and selected unbiased.

b) Risk of bias / quality appraisal

We undertook quality appraisal by categorising studies by the methodology, and the risk of bias inherent in them, as described in the Methods under *Inclusion/exclusion criteria*, above.

We were unable to appraise each study for risk of bias (RoB) as is usual for systemic reviews because of the large number of included studies, their heterogeneity of methods, and time constraints.

c) Findings

We found the evidence to address the two questions was difficult to analyse. This was largely because of the paucity of good primary studies to address them; the heterogeneous reporting methods), as well as the enormous range of animals studied; micro-organisms; and antimicrobial resistance (and methods of measuring this last outcome – ranging from culture methods to individual resistance gene identification).

Nevertheless we conclude that one study (Dutil 2010) that provides good evidence for PICO question 2: that withdrawal of the antibiotic ceftiofur resulted in a reduction of identifiable resistance in E coli and Salmonella species in retail chicken food for human consumption and humans themselves. The size of the effect is large enough to make this study credible. Moreover the time-courses are also credible: the changes in resistance prevalence change after the changes in antibiotic administration practices, and the changes in humans after that. When the antibiotic administered in the antibiotic feeds was partially re-introduced, to the resistance rates re-emerged again.

Similarly for PICO question 1, there is enough evidence to conclude that limiting antimicrobial supplementation in food animals feed reduces the burden of antimicrobial resistance. There is insufficient evidence to quantify this effect, which in any case may be specific to different antimicrobials at different doses, food animals and environments. We note the several studies that report that administration of one antimicrobial can induce resistance in an antimicrobial from a completely different class. This has wide implications.

There were other interesting observations from the literature of PICO1 detailed among the summary of results.

Conclusions

Limiting the use of antimicrobial administration to food animals is likely to reduce the presence of antimicrobial resistance in other food animals and humans. This may extend beyond the antimicrobial used to other antimicrobial classes.

More primary studies are necessary to strengthen the research evidence

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Appendix B – Tables of excluded studies

Study	Reason for exclusion
Aarestrup, F. M. (1995). "Occurrence of glycopeptide resistance among Enterococcus faecium isolates from conventional and ecological poultry farms." Microb Drug Resist 1(3): 255-257.	study type
Aarestrup, F. M., F. Bager and J. S. Andersen (2000). "Association between the use of avilamycin for growth promotion and the occurrence of resistance among Enterococcus faecium from broilers: epidemiological study and changes over time." Microb Drug Resist 6(1): 71-75.	study type
Aarestrup, F. M., A. M. Seyfarth, H. D. Emborg, K. Pedersen, R. S. Hendriksen and F. Bager (2001). "Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark." Antimicrob Agents Chemother 45(7): 2054-2059.	study type
Akwar, T. H., C. Poppe, J. Wilson, R. J. Reid-Smith, M. Dyck, J. Waddington, D. Shang, N. Dassie and S. A. McEwen (2007). "Risk factors for antimicrobial resistance among fecal Escherichia coli from residents on forty-three swine farms." Microb Drug Resist 13(1): 69-76.	study type
Alvarez-Fernandez, E., A. Cancelo, C. Diaz-Vega, R. Capita and C. Alonso-Calleja (2013). "Antimicrobial resistance in E. coli isolates from conventionally and organically reared poultry: A comparison of agar disc diffusion and Sensi Test Gram-negative methods." Food Control 30(1): 227-234.	study type
Bennedsgaard, T. W., S. M. Thamsborg, F. M. Aarestrup, C. Enevoldsen, M. Vaarst and A. B. Christoffersen (2006). "Resistance to penicillin of Staphylococcus aureus isolates from cows with high somatic cell counts in organic and conventional dairy herds in Denmark." Acta Vet Scand 48: 24.	study type
Berge, A. C., W. B. Epperson and R. H. Pritchard (2005). "Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal Escherichia coli." Vet Res 36(5-6): 723-734.	wrong outcomes
Berghash, S. R., J. N. Davidson, J. C. Armstrong and G. M. Dunny (1983). "Effects of antibiotic treatment of nonlactating dairy cows on antibiotic resistance patterns of bovine mastitis pathogens." Antimicrob Agents Chemother 24(5): 771-776.	study type
Borgen, K., G. S. Simonsen, A. Sundsfjord, Y. Wasteson, O. Olsvik and H. Kruse (2000). "Continuing high prevalence of VanA-type vancomycin-resistant enterococci on Norwegian poultry farms three years after avoparcin was banned." J Appl Microbiol 89(3): 478-485.	study type
Botelho, L. A., G. B. Kraychete, J. L. Costa e Silva, D. V. Regis, R. C. Picao, B. M. Moreira and R. R. Bonelli (2015). "Widespread distribution of CTX-M and plasmid-mediated AmpC beta-lactamases in Escherichia coli from Brazilian chicken meat." Mem Inst Oswaldo Cruz 110(2): 249-254.	study type
Boyer, T. C. and R. S. Singer (2012). "Quantitative measurement of blaCMY-2 in a longitudinal observational study of dairy cattle treated with ceftiofur." Foodborne Pathogens and Disease 9(11): 1022-1027.	relevance to PICO questions

Bunner, C. A., Norby, B., Bartlett, P. C., Erskine, R. J., Downes, F. P., & Kaneene, J. B. (2007). Prevalence and pattern of antimicrobial susceptibility in Escherichia coli isolated from pigs reared under antimicrobial-free and conventional production methods. J Am Vet Med Assoc, 231(2), 275-283. doi: 10.2460/javma.231.2.275	Study type
Buntenkoetter, V., T. Blaha, R. Tegeler, A. Fetsch, M. Hartmann, L. Kreienbrock and D. Meemken (2014). "Comparison of the phenotypic antimicrobial resistances and spa-types of methicillin-resistant Staphylococcus aureus (MRSA) isolates derived from pigs in conventional and in organic husbandry systems." Berliner Und Munchener Tierarztliche Wochenschrift 127(3-4): 135-143.	study type
Cho, S., J. B. Bender, F. Diez-Gonzalez, C. P. Fossler, C. W. Hedberg, J. B. Kaneene, P. L. Ruegg, L. D. Warnick and S. J. Wells (2006). "Prevalence and characterization of Escherichia coli O157 isolates from Minnesota dairy farms and county fairs." J Food Prot 69(2): 252-259.	no difference in exposure
Cho, S., C. P. Fossler, F. Diez-Gonzalez, S. J. Wells, C. W. Hedberg, J. B. Kaneene, P. L. Ruegg, L. D. Warnick and J. B. Bender (2007). "Antimicrobial susceptibility of Shiga toxin-producing Escherichia coli isolated from organic dairy farms, conventional dairy farms, and county fairs in Minnesota." Foodborne Pathog Dis 4(2): 178-186.	study type
Christie, P. J. and G. M. Dunny (1984). "Antibiotic selection pressure resulting in multiple antibiotic resistance and localization of resistance determinants to conjugative plasmids in streptococci." J Infect Dis 149(1): 74-82.	Study type
Ciceron, M. F. A., J. Miguel del Prado, J. Jack Echauz and E. C. Cabrera (2008). "A Comparative Study on the Antimicrobial Resistance of Escherichia coli Isolates from Chickens and Fish Grown on Integrated and Traditional Fish Farms." Philippine Agricultural Scientist 91(3): 301-307.	study type
Corbett, E. M., B. Norby, L. W. Halbert, S. T. Henderson, D. L. Grooms, S. D. Manning and J. B. Kaneene (2015). "Effect of feeding a direct-fed microbial on total and antimicrobial-resistant fecal coliform counts in preweaned dairy calves." Am J Vet Res 76(9): 780-788.	wrong exposure
Cui, S., B. Ge, J. Zheng and J. Meng (2005). "Prevalence and antimicrobial resistance of Campylobacter spp. and Salmonella serovars in organic chickens from Maryland retail stores." Appl Environ Microbiol 71(7): 4108-4111.	study type
Docic, M. and G. Bilkei (2003). "Differences in antibiotic resistance in Escherichia coli, isolated from East-European swine herds with or without prophylactic use of antibiotics." J Vet Med B Infect Dis Vet Public Health 50(1): 27-30.	study type
Dolejska, M., Z. Jurcickova, I. Literak, L. Pokludova, J. Bures, A. Hera, L. Kohoutova, J. Smola and A. Cizek (2011). "IncN plasmids carrying bla CTX-M-1 in Escherichia coli isolates on a dairy farm." Vet Microbiol 149(3-4): 513-516.	study type
Edrington, T. S., R. B. Harvey, L. A. Farrington and D. J. Nisbet (2001). "Evaluation of subtherapeutic use of the antibiotics apramycin and carbadox on the prevalence of antimicrobial-resistant Salmonella infection in swine." J Food Prot 64(12): 2067-2070.	study type

Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt and I. Nachamkin (2001). "Quinolone and macrolide resistance in Campylobacter jejuni and C-coli: Resistance mechanisms and trends in human isolates." Emerging Infectious Diseases 7(1): 24-34.	study type
European Centre for Disease Prevention and Control, European Food Safety Authority and European Medicines Agency (2015). "ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals." EFSA Journal 13(1).	no comparison
European Food Safety Authority (2009). "Joint scientific report of ECDC, EFSA and EMEA on meticillin resistant Staphylococcus aureus (MRSA) in livestock, companion animals and food." EFSA Journal 7(6).	not a study
Fraqueza, M. J., A. Martins, A. C. Borges, M. H. Fernandes, M. J. Fernandes, Y. Vaz, R. J. Bessa and A. S. Barreto (2014). "Antimicrobial resistance among Campylobacter spp. strains isolated from different poultry production systems at slaughterhouse level." Poult Sci 93(6): 1578-1586.	study type
Funk, J., T. E. Wittum, J. T. LeJeune, P. J. Rajala-Schultz, A. Bowman and A. Mack (2007). "Evaluation of stocking density and subtherapeutic chlortetracycline on Salmonella enterica subsp enterica shedding in growing swine." Veterinary Microbiology 124(3-4): 202-208.	Wrong outcomes
Gallay, A., V. Prouzet-Mauleon, I. Kempf, P. Lehours, L. Labadi, C. Camou, M. Denis, H. de Valk, J. C. Desenclos and F. Megraud (2007). "Campylobacter antimicrobial drug resistance among humans, broiler chickens, and pigs, France." Emerg Infect Dis 13(2): 259-266.	study type
Garmo, R. T., S. Waage, S. Sviland, B. I. Henriksen, O. Osteras and O. Reksen (2010). "Reproductive performance, udder health, and antibiotic resistance in mastitis bacteria isolated from Norwegian Red cows in conventional and organic farming." Acta Vet Scand 52: 11.	study type
Getachew, Y., L. Hassan, Z. Zakaria and S. A. Aziz (2013). "Genetic Variability of Vancomycin-Resistant Enterococcus faecium and Enterococcus faecalis Isolates from Humans, Chickens, and Pigs in Malaysia." Applied and Environmental Microbiology 79(15): 4528-4533.	no comparison
Gibbons, J. F., F. Boland, J. Egan, S. Fanning, B. K. Markey and F. C. Leonard (2016). "Antimicrobial Resistance of Faecal Escherichia coli Isolates from Pig Farms with Different Durations of In-feed Antimicrobial Use." Zoonoses Public Health 63(3): 241-250.	study type
Habing, G. G., J. E. Lombard, C. A. Kopral, D. A. Dargatz and J. B. Kaneene (2012). "Farm-Level Associations with the Shedding of Salmonella and Antimicrobial-Resistant Salmonella in U.S. Dairy Cattle." Foodborne Pathogens and Disease 9(9): 815-821.	study type
Halbert, L. W., J. B. Kaneene, J. Linz, L. S. Mansfield, D. Wilson, P. L. Ruegg, L. D. Warnick, S. J. Wells, C. P. Fossler, A. M. Campbell and A. M. Geiger-Zwald (2006). "Genetic mechanisms contributing to reduced tetracycline susceptibility of Campylobacter isolated from organic and conventional dairy farms	study type

in the midwestern and northeastern United States." J Food Prot 69(3): 482-488.	
Halbert, L. W., J. B. Kaneene, P. L. Ruegg, L. D. Warnick, S. J. Wells, L. S. Mansfield, C. P. Fossler, A. M. Campbell and A. M. Geiger-Zwald (2006). "Evaluation of antimicrobial susceptibility patterns in Campylobacter spp isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States." J Am Vet Med Assoc 228(7): 1074-1081.	study type
Hammerum, A. M., J. Larsen, V. D. Andersen, C. H. Lester, T. S. Skovgaard Skytte, F. Hansen, S. S. Olsen, H. Mordhorst, R. L. Skov, F. M. Aarestrup and Y. Agerso (2014). "Characterization of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins." J Antimicrob Chemother 69(10): 2650-2657.	study type
Han, F., S. I. Lestari, S. Pu and B. Ge (2009). "Prevalence and antimicrobial resistance among Campylobacter spp. in Louisiana retail chickens after the enrofloxacin ban." Foodborne Pathog Dis 6(2): 163-171.	study type
Hershberger, E., S. F. Oprea, S. M. Donabedian, M. Perri, P. Bozigar, P. Bartlett and M. J. Zervos (2005). "Epidemiology of antimicrobial resistance in enterococci of animal origin." J Antimicrob Chemother 55(1): 127-130.	study type
Heuer, O. E., K. Pedersen, L. B. Jensen, M. Madsen and J. E. Olsen (2002b). "Persistence of vancomycin-resistant enterococci (VRE) in broiler houses after the avoparcin ban." Microb Drug Resist 8(4): 355-361.	study type
Hille, K., J. Fischer, L. Falgenhauer, H. Sharp, G. M. Brenner, K. Kadlec, A. Friese, S. Schwarz, C. Imirzalioglu, M. Kietzmann, C. Von Munchhausen and L. Kreienbrock (2014). "[On the occurence of extended-spectrum- and AmpC-beta-lactamase-producing Escherichia coli in livestock: results of selected European studies]." Berl Munch Tierarztl Wochenschr 127(9-10): 403-411.	study type
Huijbers, P. M., A. H. van Hoek, E. A. Graat, A. P. Haenen, A. Florijn, P. D. Hengeveld and E. van Duijkeren (2015). "Methicillin-resistant Staphylococcus aureus and extended-spectrum and AmpC beta-lactamase-producing Escherichia coli in broilers and in people living and/or working on organic broiler farms." Vet Microbiol 176(1-2): 120-125.	study type
Iliadis, N. (1992). "The selection of Salmonella gallinarum, Salmonella pullorum and Escherichia coli in chickens after chloramphenicol administration." Berliner und Münchener tierärztliche Wochenschrift 105(11): 383-386.	study type
Jackson, C. R., P. J. Fedorka-Cray, J. B. Barrett and S. R. Ladely (2004). "Effects of tylosin use on erythromycin resistance in enterococci isolated from swine." Applied and Environmental Microbiology 70(7): 4205-4210.	study type

Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, D. G. Renter and T. G. Nagaraja (2008). "Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle." J Anim Sci 86(5): 1182-1190.	study type
Johnson, J. R., M. R. Sannes, C. Croy, B. Johnston, C. Clabots, M. A. Kuskowski, J. Bender, K. E. Smith, P. L. Winokur and E. A. Belongia (2007). "Antimicrobial drug-resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002-2004." Emerg Infect Dis 13(6): 838-846.	study type
Jones, F. T., B. E. Langlois, G. L. Cromwell and V. W. Hays (1983). "Effect of feeding chlortetracycline or virginiamycin on shedding of salmonellae from experimentally-infected swine." J Anim Sci 57(2): 279-285.	wrong outcomes
Juntunen, P., S. Olkkola and M. L. Hanninen (2011). "Longitudinal on-farm study of the development of antimicrobial resistance in Campylobacter coli from pigs before and after danofloxacin and tylosin treatments." Vet Microbiol 150(3-4): 322-330.	Wrong population
Kilonzo-Nthenge, A., A. Brown, S. N. Nahashon and D. Long (2015). "Occurrence and antimicrobial resistance of enterococci isolated from organic and conventional retail chicken." J Food Prot 78(4): 760-766.	study type
Klare, I., D. Badstubner, C. Konstabel, G. Bohme, H. Claus and W. Witte (1999). "Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry." Microbial Drug Resistance-Mechanisms Epidemiology and Disease 5(1): 45-52.	study type
Lazarus, B., D. L. Paterson, J. L. Mollinger and B. A. Rogers (2015). "Do Human Extraintestinal Escherichia coli Infections Resistant to Expanded-Spectrum Cephalosporins Originate From Food-Producing Animals? A Systematic Review." Clinical Infectious Diseases 60(3): 439-452.	Wrong outcomes (in studies included in the systematic review)
Levy, S. B. (1978). "Emergence of antibiotic-resistant bacteria in the intestinal flora of farm inhabitants." J Infect Dis 137(5): 689-690.	Summary data not longitudinal
Linton, A. H., A. J. Hedges and P. M. Bennett (1988). "Monitoring for the development of antimicrobial resistance during the use of olaquindox as a feed additive on commercial pig farms." J Appl Bacteriol 64(4): 311-327.	Study type
Looft, T., T. A. Johnson, H. K. Allen, D. O. Bayles, D. P. Alt, R. D. Stedtfeld, W. J. Sul, T. M. Stedtfeld, B. L. Chai, J. R. Cole, S. A. Hashsham, J. M. Tiedje and T. B. Stanton (2012). "In-feed antibiotic effects on the swine intestinal microbiome." Proceedings of the National Academy of Sciences of the United States of America 109(5): 1691-1696.	relevance to PICO questions
Luangtongkum, T., T. Y. Morishita, A. J. Ison, S. Huang, P. F. McDermott and Q. Zhang (2006). "Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of Campylobacter spp. in poultry." Appl Environ Microbiol 72(5): 3600-3607.	study type

Lutz, E. A., M. J. McCarty, D. F. Mollenkopf, J. A. Funk, W. A. Gebreyes and T. E. Wittum (2011). "Ceftiofur use in finishing swine barns and the recovery of fecal Escherichia coli or Salmonella spp. resistant to ceftriaxone." Foodborne Pathog Dis 8(11): 1229-1234.	study type
Makita, K., M. Goto, M. Ozawa, M. Kawanishi, R. Koike, T. Asai and Y. Tamura (2016). "Multivariable Analysis of the Association Between Antimicrobial Use and Antimicrobial Resistance in Escherichia coli Isolated from Apparently Healthy Pigs in Japan." Microbial Drug Resistance 22(1): 28-39.	study type
Mamber, S. W. and S. E. Katz (1985). "Effects of antimicrobial agents fed to chickens on some gram-negative enteric bacilli." Appl Environ Microbiol 50(3): 638-648.	study type
Martin, S. W., A. H. Meek and R. A. Curtis (1983). "Antimicrobial use in feedlot calves: its association with culture rates and antimicrobial susceptibility." Can J Comp Med 47(1): 6-10.	study type
Mathew, A. G., M. A. Beckmann and A. M. Saxton (2001). "A comparison of antibiotic resistance in bacteria isolated from swine herds in which antibiotics were used or excluded." Journal of Swine Health and Production 9(3): 125-129.	study type
Menzies-Gow, N. J. and N. J. Young (2011). "Antibiotic resistance in faecal bacteria isolated from horses receiving virginiamycin for the prevention of pasture-associated laminitis." Veterinary Microbiology 152(3-4): 424-428.	study type
Mercer, H. D., D. Pocurull, S. Gaines, S. Wilson and J. V. Bennett (1971). "Characteristics of antimicrobial resistance of Escherichia coli from animals: relationship to veterinary and management uses of antimicrobial agents." Appl Microbiol 22(4): 700-705.	study type
Moniri, R. and K. Dastehgoli (2005). "Fluoroquinolone-resistant Escherichia coli isolated from healthy broilers with previous exposure to fluoroquinolones: Is there a link?" Microbial Ecology in Health and Disease 17(2): 69-74.	study type
Neyra, R. C., J. A. Frisancho, J. L. Rinsky, C. Resnick, K. C. Carroll, A. M. Rule, T. Ross, Y. You, L. B. Price and E. K. Silbergeld (2014). "Multidrug-resistant and methicillin-resistant Staphylococcus aureus (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA)." Environ Health Perspect 122(5): 471-477.	study type
Park, Y. K., L. K. Fox, D. D. Hancock, W. McMahan and Y. H. Park (2012). "Prevalence and antibiotic resistance of mastitis pathogens isolated from dairy herds transitioning to organic management." J Vet Sci 13(1): 103-105.	study type
Pereira, R. V., T. M. Santos, M. L. Bicalho, L. S. Caixeta, V. S. Machado and R. C. Bicalho (2011). "Antimicrobial resistance and prevalence of virulence factor genes in fecal Escherichia coli of Holstein calves fed milk with and without antimicrobials." J Dairy Sci 94(9): 4556-4565.	study type
Pereira, R. V., J. D. Siler, J. C. Ng, M. A. Davis, Y. T. Grohn and L. D. Warnick (2014). "Effect of on-farm use of antimicrobial drugs on resistance in fecal Escherichia coli of preweaned dairy calves." J Dairy Sci 97(12): 7644-7654.	study type
Petersen, A. and A. Dalsgaard (2003). "Antimicrobial resistance of intestinal Aeromonas spp. and Enterococcus spp. in fish cultured	study type

in integrated broiler-fish farms in Thailand." Aquaculture 219(1-4): 71-82.	
Petersen, A. and A. Dalsgaard (2003). "Species composition and antimicrobial resistance genes of Enterococcus spp, isolated from integrated and traditional fish farms in Thailand." Environ Microbiol 5(5): 395-402.	study type
Pupavac, V., V. Juric, M. Ristic and S. Filipovic (1996). "The effect of including additives in feeds for pigs on the appearance of resistance in E-coli." Acta Veterinaria-Beograd 46(1): 45-49.	study type
Reinstein, S., J. T. Fox, X. Shi, M. J. Alam, D. G. Renter and T. G. Nagaraja (2009). "Prevalence of Escherichia coli O157:H7 in organically and naturally raised beef cattle." Applied and Environmental Microbiology 75(16): 5421-5423.	no information on difference in exposure
Roesch, M., V. Perreten, M. G. Doherr, W. Schaeren, M. Schallibaum and J. W. Blum (2006). "Comparison of antibiotic resistance of udder pathogens in dairy cows kept on organic and on conventional farms." J Dairy Sci 89(3): 989-997.	study type
Rollo, S. N., B. Norby, P. C. Bartlett, H. M. Scott, D. L. Wilson, V. R. Fajt, J. E. Linz, C. E. Bunner, J. B. Kaneene and J. C. Huber, Jr. (2010). "Prevalence and patterns of antimicrobial resistance in Campylobacter spp isolated from pigs reared under antimicrobial-free and conventional production methods in eight states in the Midwestern United States." J Am Vet Med Assoc 236(2): 201-210.	study type
Rood, J. I., J. R. Buddle, A. J. Wales and R. Sidhu (1985). "The occurrence of antibiotic resistance in Clostridium perfringens from pigs." Aust Vet J 62(8): 276-279.	study type
Rosengren, L. B., C. L. Waldner, R. J. Reid-Smith, P. M. Dowling and J. C. Harding (2007). "Associations between feed and water antimicrobial use in farrow-to-finish swine herds and antimicrobial resistance of fecal Escherichia coli from grow-finish pigs." Microb Drug Resist 13(4): 261-269.	Study type
Sapkota, A. R., R. M. Hulet, G. Zhang, P. McDermott, E. L. Kinney, K. J. Schwab and S. W. Joseph (2011). "Lower prevalence of antibiotic-resistant Enterococci on U.S. conventional poultry farms that transitioned to organic practices." Environ Health Perspect 119(11): 1622-1628.	study type
Sato, K., P. C. Bartlett and M. A. Saeed (2005). "Antimicrobial susceptibility of Escherichia coli isolates from dairy farms using organic versus conventional production methods." J Am Vet Med Assoc 226(4): 589-594.	study type
Sato, T., T. Okubo, M. Usui, S.-i. Yokota, S. Izumiyama and Y. Tamura (2014). "Association of Veterinary Third-Generation Cephalosporin Use with the Risk of Emergence of Extended-Spectrum-Cephalosporin Resistance in Escherichia coli from Dairy Cattle in Japan." Plos One 9(4).	study type
Savasan, S., A. Ciftci and K. S. Diker (2004). "Emergence of quinolone resistance among chicken isolates of Campylobacter in Turkey." Turkish Journal of Veterinary & Animal Sciences 28(2): 391-397.	study type
Schmidt, J. W., D. Griffin, L. A. Kuehn and D. M. Brichta-Harhay (2013). "Influence of therapeutic ceftiofur treatments of feedlot cattle on fecal and hide prevalences of commensal Escherichia coli resistant to expanded-spectrum cephalosporins, and molecular	no difference in exposure

characterization of resistant isolates." Applied and Environmental Microbiology 79(7): 2273-2283.	
Schwaiger, K., J. Bauer and C. S. Holzel (2013). "Selection and persistence of antimicrobial-resistant Escherichia coli including extended-spectrum beta-lactamase producers in different poultry flocks on one chicken farm." Microb Drug Resist 19(6): 498-506.	no difference in exposure
Schwaiger, K., E. M. Schmied and J. Bauer (2010). "Comparative analysis on antibiotic resistance characteristics of Listeria spp. and Enterococcus spp. isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany." Zoonoses Public Health 57(3): 171-180.	study type
Scott, H. M., L. D. Campbell, R. B. Harvey, K. M. Bischoff, W. Q. Alali, K. S. Barling and R. C. Anderson (2005). "Patterns of antimicrobial resistance among commensal Escherichia coli isolated from integrated multi-site housing and worker cohorts of humans and swine." Foodborne Pathogens and Disease 2(1): 24-37.	study type
Siegel, D., W. G. Huber and S. Drysdale (1975). "Human therapeutic and agricultural uses of antibacterial drugs and resistance of the enteric flora of humans." Antimicrob Agents Chemother 8(5): 538-543.	study type
Siemon, C. E., P. B. Bahnson and W. A. Gebreyes (2007). "Comparative investigation of prevalence and antimicrobial resistance of Salmonella between pasture and conventionally reared poultry." Avian Dis 51(1): 112-117.	study type
Simoneit, C., E. Burow, B. A. Tenhagen and A. Kaesbohrer (2015). "Oral administration of antimicrobials increase antimicrobial resistance in E-coli from chicken - A systematic review." Preventive Veterinary Medicine 118(1): 1-7.	No additional relevant studies (beyond those identified in the literature search)
Singer, R. S., S. K. Patterson and R. L. Wallace (2008). "Effects of Therapeutic Ceftiofur Administration to Dairy Cattle on Escherichia coli Dynamics in the Intestinal Tract." Applied and Environmental Microbiology 74(22): 6956-6962.	Wrong population
Smith, J. L., D. J. Drum, Y. Dai, J. M. Kim, S. Sanchez, J. J. Maurer, C. L. Hofacre and M. D. Lee (2007). "Impact of antimicrobial usage on antimicrobial resistance in commensal Escherichia coli strains colonizing broiler chickens." Appl Environ Microbiol 73(5): 1404-1414.	study type
Sorum, M., G. Holstad, A. Lillehaug and H. Kruse (2004). "Prevalence of vancomycin resistant enterococci on poultry farms established after the ban of avoparcin." Avian Dis 48(4): 823-828.	Study type
Swezey, J. L., B. B. Baldwin and M. C. Bromel (1981). "Effect of oxytetracycline as a turkey feed additive." Poult Sci 60(4): 738-743.	no summary data available
Tadesse, D. A., P. B. Bahnson, J. A. Funk, S. Thakur, W. E. Morrow, T. Wittum, F. DeGraves, P. Rajala-Schultz and W. A. Gebreyes (2011). "Prevalence and antimicrobial resistance profile of Campylobacter spp. isolated from conventional and antimicrobial-free swine production systems from different U.S. regions." Foodborne Pathog Dis 8(3): 367-374.	study type

Tamang, M. D., M. Gurung, H. M. Nam, D. C. Moon, S. R. Kim, G. C. Jang, D. Y. Jung, S. C. Jung, Y. H. Park and S. K. Lim (2015). "Prevalence and characterization of Salmonella in pigs from conventional and organic farms and first report of S. serovar 1,4,[5],12:i:- from Korea." Vet Microbiol 178(1-2): 119-124.	study type
Thakur, S. and W. A. Gebreyes (2005). "Prevalence and antimicrobial resistance of Campylobacter in antimicrobial-free and conventional pig production systems." J Food Prot 68(11): 2402-2410.	study type
Tikofsky, L. L., J. W. Barlow, C. Santisteban and Y. H. Schukken (2003). "A comparison of antimicrobial susceptibility patterns for Staphylococcus aureus in organic and conventional dairy herds." Microb Drug Resist 9 Suppl 1: S39-45.	study type
Tragesser, L. A., T. E. Wittum, J. A. Funk, P. L. Winokur and P. J. Rajala-Schultz (2006). "Association between ceftiofur use and isolation of Escherichia coli with reduced susceptibility to ceftriaxone from fecal samples of dairy cows." Am J Vet Res 67(10): 1696-1700.	study type
Unicomb, L. E., J. Ferguson, R. J. Stafford, R. Ashbolt, M. D. Kirk, N. G. Becker, M. S. Patel, G. L. Gilbert, M. Valcanis and L. Mickan (2006). "Low-level fluoroquinolone resistance among Campylobacter jejuni isolates in Australia." Clin Infect Dis 42(10): 1368-1374.	study type
van den Bogaard, A. E., N. London, C. Driessen and E. E. Stobberingh (2001). "Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers." J Antimicrob Chemother 47(6): 763-771.	study type
van den Bogaard, A. E., R. Willems, N. London, J. Top and E. E. Stobberingh (2002). "Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers." J Antimicrob Chemother 49(3): 497-505.	study type
Varela, N. P., P. Gadbois, C. Thibault, M. Gottschalk, P. Dick and J. Wilson (2013). "Antimicrobial resistance and prudent drug use for Streptococcus suis." Anim Health Res Rev 14(1): 68-77.	study type
Vinayagamoorthy, T. (1987). "Mobilization of antibiotic resistance genes among farm animals and human hosts in a developing country (Sri Lanka)." Singapore Med J 28(2): 134-139.	study type
Wilhelm, B., A. Rajic, L. Waddell, S. Parker, J. Harris, K. C. Roberts, R. Kydd, J. Greig and A. Baynton (2009). "Prevalence of zoonotic or potentially zoonotic bacteria, antimicrobial resistance, and somatic cell counts in organic dairy production: current knowledge and research gaps." Foodborne Pathog Dis 6(5): 525-539.	study type

Appendix C – Search strategies

PubMed: Antibiotic resistance in food animals

("Drug Resistance, Microbial"[Mesh] OR “Drug resistance”[tiab] OR “Drug resistant”[tiab] OR “multidrug-resistant”[tiab] OR “multidrug resistant”[tiab] OR “Microbial resistant”[tiab] OR “Microbial resistance”[tiab] OR “Antibiotic resistance”[tiab] OR “Antibiotic resistant”[tiab] OR “Antibacterial resistance”[tiab] OR “Antibacterial resistant”[tiab] OR “Antimicrobial resistance”[tiab] OR “Antimicrobial resistant”[tiab])
AND
("Anti-Bacterial Agents"[Mesh] OR "Macrolides"[Mesh] OR "beta-Lactams"[Mesh] OR Antibacterial[tiab] OR Antibacterials[tiab] OR Antibiotics[tiab] OR Antibiotic[tiab] OR Macrolides[tiab] OR Macrolide[tiab] OR beta-Lactams[tiab] OR Antimicrobial[tiab] OR Antimicrobials[tiab] OR Penicillin[tiab] OR Methicillin[tiab] OR Ampicillin[tiab] OR Azithromycin[tiab] OR Cephalexin[tiab] OR Apramycin[tiab] OR Streptomycin[tiab])
AND
("Cattle"[Mesh] OR "Swine"[Mesh] OR “Poultry”[Mesh] OR “Farm animals”[tiab] OR “Food animals”[tiab] OR Cattle[tiab] OR Beef[tiab] OR Swine[tiab] OR Pig[tiab] OR Pigs[tiab] OR Poultry[tiab] OR Chickens[tiab])
AND
(“Systematic review”[tiab] OR “Meta-analysis”[tiab] OR Randomised[tiab] OR Randomized[tiab] OR Randomise[tiab] OR Randomize[tiab] OR Randomly[tiab] OR Controlled[tiab] OR “Control group”[tiab] OR Epidemiology[sh] OR"Morbidity"[Mesh] OR Comparison[tiab] OR Compared[tiab] OR Compare[tiab] OR Examined[tiab] OR Observations[tiab] OR Observed[tiab] OR Longitudinal[tiab] OR Experimental[tiab] OR Experiments[tiab] OR Investigate[tiab] OR (Before[tiab] AND After[tiab]))
AND
("Microbial Sensitivity Tests"[Mesh] OR Isolates[tiab] OR Isolated[tiab] OR Samples[tiab] OR Strains[tiab] OR Strain[tiab] OR Carriage[tiab] OR Phylogenetic OR Metagenomic OR PCR)
AND
("Zoonoses"[Mesh] OR "Animal Diseases"[Mesh] OR Zoonoses[tiab] OR Transmitted[tiab] OR Transmission[tiab] OR Susceptibility[tiab] OR Susceptible[tiab] OR Selective[tiab] OR Selection[tiab])
NOT
("Humans"[Mesh])

PubMed: Transfer of antibiotic resistance from food animals to humans

("Drug Resistance, Microbial"[Mesh] OR “Drug resistance”[tiab] OR “Drug resistant”[tiab] OR “multidrug-resistant”[tiab] OR “multidrug resistant”[tiab] OR “Microbial resistant”[tiab] OR “Microbial resistance”[tiab] OR “Antibiotic resistance”[tiab] OR “Antibiotic resistant”[tiab] OR “Antibacterial resistance”[tiab] OR “Antibacterial resistant”[tiab] OR “Antimicrobial resistance”[tiab] OR “Antimicrobial resistant”[tiab])
AND
("Anti-Bacterial Agents"[Mesh] OR "Macrolides"[Mesh] OR "beta-Lactams"[Mesh] OR Antibacterial[tiab] OR Antibacterials[tiab] OR Antibiotics[tiab] OR Antibiotic[tiab] OR Macrolides[tiab] OR Macrolide[tiab] OR beta-Lactams[tiab] OR Antimicrobial[tiab] OR Antimicrobials[tiab] OR Penicillin[tiab] OR Methicillin[tiab] OR Ampicillin[tiab] OR Azithromycin[tiab] OR Cephalexin[tiab] OR Apramycin[tiab] OR Streptomycin[tiab])
AND
("Cattle"[Mesh] OR "Swine"[Mesh] OR “Poultry”[Mesh] OR “Farm animals”[tiab] OR “Food animals”[tiab] OR Cattle[tiab] OR Beef[tiab] OR Swine[tiab] OR Pig[tiab] OR Pigs[tiab] OR Poultry[tiab] OR Chickens[tiab])
AND
(“Systematic review”[tiab] OR “Meta-analysis”[tiab] OR Randomised[tiab] OR Randomized[tiab] OR Randomise[tiab] OR Randomize[tiab] OR Randomly[tiab] OR Controlled[tiab] OR “Control group”[tiab] OR Epidemiology[sh] OR"Morbidity"[Mesh] OR Comparison[tiab] OR Compared[tiab] OR Compare[tiab] OR Examined[tiab] OR Observations[tiab] OR Observed[tiab] OR Longitudinal[tiab] OR Experimental[tiab] OR Experiments[tiab] OR Investigate[tiab] OR (Before[tiab] AND After[tiab]))

AND
("Microbial Sensitivity Tests"[Mesh] OR Isolates[tiab] OR Isolated[tiab] OR Samples[tiab] OR Strains[tiab] OR Strain[tiab] OR Carriage[tiab] OR Phylogenetic OR Metagenomic OR PCR)
AND
("Zoonoses"[Mesh] OR "Animal Diseases"[Mesh] OR Zoonoses[tiab] OR Transmitted[tiab] OR Transmission[tiab] OR Susceptibility[tiab] OR Susceptible[tiab] OR Selective[tiab] OR Selection[tiab])
AND
("Humans"[Mesh] OR Humans[tiab] OR Human[tiab] OR Farmer[tiab] OR Farmers[tiab] OR “Farm worker”[tiab] OR “Farm workers”[tiab])

Embase: Antibiotic resistance in food animals
((‘antibiotic resistance'/exp OR "Drug resistance":ti,ab OR "Drug resistant":ti,ab OR multidrug-resistant:ti,ab OR "multidrug resistant":ti,ab OR "Microbial resistant":ti,ab OR "Microbial resistance":ti,ab OR "Antibiotic resistance":ti,ab OR "Antibiotic resistant":ti,ab OR "Antibacterial resistance":ti,ab OR "Antibacterial resistant":ti,ab OR "Antimicrobial resistance":ti,ab OR "Antimicrobial resistant":ti,ab))
AND
(('antiinfective agent'/exp OR 'macrolide'/exp OR 'beta lactam'/exp OR Antibacteria:ti,ab OR Antibacterials:ti,ab OR Antibiotics:ti,ab OR Antibiotic:ti,ab OR Macrolides:ti,ab OR Macrolide:ti,ab OR beta-Lactams:ti,ab OR Antimicrobial:ti,ab OR Antimicrobials:ti,ab OR Penicillin:ti,ab OR Methicillin:ti,ab OR Ampicillin:ti,ab OR Azithromycin:ti,ab OR Cephalexin:ti,ab OR Apramycin:ti,ab OR Streptomycin:ti,ab))
AND
(('bovine'/exp OR 'pig'/exp OR 'poultry'/exp OR "Farm animals":ti,ab OR "Food animals":ti,ab OR Cattle:ti,ab OR Beef:ti,ab OR Swine:ti,ab OR Pig:ti,ab OR Pigs:ti,ab OR Poultry:ti,ab OR Chickens:ti,ab))
AND
('Systematic review":ti,ab OR Meta-analysis:ti,ab OR Randomised:ti,ab OR Randomized:ti,ab OR Randomise:ti,ab OR Randomize:ti,ab OR Randomly:ti,ab OR Controlled:ti,ab OR "Control group":ti,ab OR 'morbidity'/exp OR Comparison:ti,ab OR Compared:ti,ab OR Compare:ti,ab OR Examined:ti,ab OR Observations:ti,ab OR Observed:ti,ab OR Longitudinal:ti,ab OR Experimental:ti,ab OR Experiments:ti,ab OR Investigate:ti,ab OR (Before:ti,ab AND After:ti,ab))
AND
(('microbial sensitivity test'/exp OR Isolates:ti,ab OR Isolated:ti,ab OR Samples:ti,ab OR Strains:ti,ab OR Strain:ti,ab OR Carriage:ti,ab OR Phylogenetic OR Metagenomic OR PCR))
AND
(('zoonosis'/exp OR 'animal disease'/exp OR Zoonoses:ti,ab OR Transmitted:ti,ab OR Transmission:ti,ab OR Susceptibility:ti,ab OR Susceptible:ti,ab OR Selective:ti,ab OR Selection:ti,ab))
(NOT)
('human'/exp)

Embase: Transfer of antibiotic resistance from food animals to humans
((‘antibiotic resistance'/exp OR "Drug resistance":ti,ab OR "Drug resistant":ti,ab OR multidrug-resistant:ti,ab OR "multidrug resistant":ti,ab OR "Microbial resistant":ti,ab OR "Microbial resistance":ti,ab OR "Antibiotic resistance":ti,ab OR "Antibiotic resistant":ti,ab OR "Antibacterial resistance":ti,ab OR "Antibacterial resistant":ti,ab OR "Antimicrobial resistance":ti,ab OR "Antimicrobial resistant":ti,ab))
AND
(('antiinfective agent'/exp OR 'macrolide'/exp OR 'beta lactam'/exp OR Antibacteria:ti,ab OR Antibacterials:ti,ab OR Antibiotics:ti,ab OR Antibiotic:ti,ab OR Macrolides:ti,ab OR Macrolide:ti,ab OR beta-Lactams:ti,ab OR Antimicrobial:ti,ab OR Antimicrobials:ti,ab OR Penicillin:ti,ab OR Methicillin:ti,ab OR Ampicillin:ti,ab OR Azithromycin:ti,ab OR Cephalexin:ti,ab OR Apramycin:ti,ab OR Streptomycin:ti,ab))
AND
(('bovine'/exp OR 'pig'/exp OR 'poultry'/exp OR "Farm animals":ti,ab OR "Food animals":ti,ab OR Cattle:ti,ab OR Beef:ti,ab OR Swine:ti,ab OR Pig:ti,ab OR Pigs:ti,ab OR Poultry:ti,ab OR Chickens:ti,ab))
AND
('Systematic review":ti,ab OR Meta-analysis:ti,ab OR Randomised:ti,ab OR Randomized:ti,ab OR Randomise:ti,ab OR Randomize:ti,ab OR Randomly:ti,ab OR Controlled:ti,ab OR "Control group":ti,ab OR 'morbidity'/exp OR Comparison:ti,ab OR Compared:ti,ab OR Compare:ti,ab OR Examined:ti,ab OR

Observations:ti,ab OR Observed:ti,ab OR Longitudinal:ti,ab OR Experimental:ti,ab OR Experiments:ti,ab OR Investigate:ti,ab OR (Before:ti,ab AND After:ti,ab))
AND
(('microbial sensitivity test'/exp OR Isolates:ti,ab OR Isolated:ti,ab OR Samples:ti,ab OR Strains:ti,ab OR Strain:ti,ab OR Carriage:ti,ab OR Phylogenetic OR Metagenomic OR PCR))
AND
(('zoonosis'/exp OR 'animal disease'/exp OR Zoonoses:ti,ab OR Transmitted:ti,ab OR Transmission:ti,ab OR Susceptibility:ti,ab OR Susceptible:ti,ab OR Selective:ti,ab OR Selection:ti,ab))
AND
(('human'/exp OR Humans:ti,ab OR Human:ti,ab OR Farmer:ti,ab OR Farmers:ti,ab OR "Farm worker":ti,ab OR "Farm workers":ti,ab))

Web of Science: Antibiotic resistance in food animals
("Drug resistance" OR "Drug resistant" OR "multidrug-resistant" OR "multidrug resistant" OR "Microbial resistant" OR "Microbial resistance" OR "Antibiotic resistance" OR "Antibiotic resistant" OR "Antibacterial resistance" OR "Antibacterial resistant" OR "Antimicrobial resistance" OR "Antimicrobial resistant")
AND
(Antibacterial OR Antibacterials OR Antibiotics OR Antibiotic OR Macrolides OR Macrolide OR beta-Lactams OR Antimicrobial OR Antimicrobials OR Penicillin OR Methicillin OR Ampicillin OR Azithromycin OR Cephalexin OR Apramycin OR Streptomycin)
AND
("Farm animals" OR "Food animals" OR Cattle OR Beef OR Swine OR Pig OR Pigs OR Poultry OR Chickens)
AND
("Systematic review" OR "Meta-analysis" OR Randomised OR Randomized OR Randomise OR Randomize OR Randomly OR Controlled OR "Control group" OR Comparison OR Compared OR Compare OR Examined OR Observations OR Observed OR Longitudinal OR Experimental OR Experiments OR Investigate OR (Before AND After))
AND
(Isolates OR Isolated OR Samples OR Strains OR Strain OR Carriage OR Phylogenetic OR Metagenomic OR PCR)
AND
(Zoonoses OR Transmitted OR Transmission OR Susceptibility OR Susceptible OR Selective OR Selection)
NOT
(Humans OR Human)

Web of Science: Transfer of antibiotic resistance from food animals to humans
("Drug resistance" OR "Drug resistant" OR "multidrug-resistant" OR "multidrug resistant" OR "Microbial resistant" OR "Microbial resistance" OR "Antibiotic resistance" OR "Antibiotic resistant" OR "Antibacterial resistance" OR "Antibacterial resistant" OR "Antimicrobial resistance" OR "Antimicrobial resistant")
AND
(Antibacterial OR Antibacterials OR Antibiotics OR Antibiotic OR Macrolides OR Macrolide OR beta-Lactams OR Antimicrobial OR Antimicrobials OR Penicillin OR Methicillin OR Ampicillin OR Azithromycin OR Cephalexin OR Apramycin OR Streptomycin)
AND
("Farm animals" OR "Food animals" OR Cattle OR Beef OR Swine OR Pig OR Pigs OR Poultry OR Chickens)
AND
("Systematic review" OR "Meta-analysis" OR Randomised OR Randomized OR Randomise OR Randomize OR Randomly OR Controlled OR "Control group" OR Comparison OR Compared OR Compare OR Examined OR Observations OR Observed OR Longitudinal OR Experimental OR Experiments OR Investigate OR (Before AND After))
AND
(Isolates OR Isolated OR Samples OR Strains OR Strain OR Carriage OR Phylogenetic OR Metagenomic OR PCR)

AND
(Zoonoses OR Transmitted OR Transmission OR Susceptibility OR Susceptible OR Selective OR Selection)
AND
(Humans OR Human OR Farmer OR Farmers OR "Farm worker" OR "Farm workers

1.2 Restriction in the use of antibiotics in food animals and antibiotic resistance in food animals and humans – a systematic review and meta-analysis (University of Calgary, Canada)

Authors	Karen L. Tang, Niamh P. Caffrey, Diego B. Nóbrega, Susan C. Cork, Paul E. Ronksley, Herman W. Barkema, Alicia J. Polachek, Heather Ganshorn, Nishan Sharma, James D. Kellner, William A. Ghali
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Submission	Revised in March 2017
Journal Publication	Tang KL, Caffrey NP, Nobrega DB, Cork SC, Ronksley PE, Barkema HW et al. Restriction in the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and humans – a systematic review and meta-analysis. Lancet Planetary Health. 2017; In Press.

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Abstract

Background: Antibiotics are the cornerstone of therapy for bacterial infectious diseases in humans and animals. The One Health approach recognizes that the health of humans, animals, and the environment are intricately linked, that the use of antibiotics in animals select for resistant bacteria, and that bacteria and their resistant genetic elements can be transmitted cross-species from animals to humans. The rise in resistance to antibiotics is therefore a threat to public health globally and there is a growing recognition that we may need to use antibacterial agents in a more judicious way. In this systematic review and meta-analysis, commissioned by the World Health Organization, we sought to summarize the evidence on the effect that interventions that reduce antibiotic use in food animals has on the presence of antibiotic resistant bacteria and resistant genetic elements in animals and in humans.

Methods: We conducted a comprehensive search of electronic databases (including Agricola, AGRIS, BIOSIS Previews, CAB Abstracts, MEDLINE, EMBASE, Global Index Medicus, ProQuest Dissertations, and Science Citation Index) in July 2016, with an update in January 2017. In addition, we reviewed conference proceedings of major scientific meetings on antibiotic resistance and conducted a thorough grey literature search that included governmental websites from a wide range of regions globally. Inclusion criteria were original studies that reported on any interventions that aimed to reduce antibiotic use in food animals and compared presence of antibiotic resistant bacteria or genetic resistance elements between intervention and comparator groups in food animals or in humans. Analysis was conducted and reported separately for animals and humans. We pooled studies that reported an absolute risk difference in the prevalence of resistance in bacteria isolated from intervention compared to control groups using DerSimonian and Laird random-effects models. Meta-analysis for animals was conducted separately for different antibiotic classes for six different bacteria and sample type combinations, while meta-analysis for humans was not stratified due to smaller numbers of studies. Studies reporting on genetic elements of resistance and studies that could not be meta-analyzed (because they reported on different units of analyses or did not provide risk differences) were described qualitatively.

Results: A total of 5,945 unique records were identified and screened. Of these, 386 were reviewed at the full-text stage. In total, 181 studies were included in the systematic review. Of these, 179 described antibiotic resistance outcomes in animals, of which 81 were meta-analyzed. Twenty-one studies described antibiotic resistance outcomes in humans (19 of which also reported antibiotic resistance in animals), of which 13 were meta-analyzed. The pooled absolute risk reduction of the prevalence of antibiotic resistance in animals, with interventions that restricted antibiotic use, varied across different antibiotic classes, bacteria, and sample types, but ranged from 0% to 39%; in general, the prevalence of antibiotic resistance was commonly 10-20% lower in intervention compared to control groups. The pooled prevalence of multi-drug resistance was 24-32% lower in bacteria isolated from intervention groups. These findings held through many different layers of stratification including by intervention type. Similarly, for humans, the pooled prevalence of antibiotic resistance was 24% lower in intervention groups (where interventions to reduce antibiotic use in food animals were implemented) compared to control groups. The effect was similar, albeit weaker, when considering humans without direct contact with livestock animals, compared to farm workers.

Conclusion: There is a large body of evidence that, when pooled, consistently shows that interventions that restrict the use of antibiotics in food animals are associated with a reduction in the presence of antibiotic resistant bacteria in these animals. Our analysis also suggests that there may be a reduction in the number of antibiotic resistant bacteria in human populations with these interventions, with the greatest effect for those in direct contact with animals. These findings are in keeping with One Health phenomena and the understanding that animals and humans share the same environment, and they suggest that the effects of restricting antibiotic use in animals on antibiotic resistance may extend beyond the animals themselves.

Abbreviations

AGISAR	Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AGP	antimicrobial growth promoter
AMR	antimicrobial resistance
AMU	antimicrobial use
CI	confidence interval
CIA	Critically Important Antimicrobials
DALY	disability adjusted life years
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
FAO	Food and Agriculture Organization
GRADE	grading of recommendations assessment, development and evaluation
MARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
MeSH	medical subject heading
MIC	minimum inhibitory concentration
MRSA	methicillin resistant *Staphylococcus aureus*
N/A	not application
NR	not reported
OIE	World Organization of Animal Health
OR	odds ratio
PICOD	populations, intervention, control/comparator, outcome, design
PRISMA	preferred reporting items for systematic reviews and meta-analyses
RAB	reduced antibiotic farms
RAB-CD	reduced antibiotic with cleaning and disinfection protocol farms
RCT	randomized controlled trials
RD	risk difference
UK	United Kingdom
USA	United States
WHO	World Health Organization
WHO FERG	World Health Organization Foodborne Diseases Epidemiology Reference Group

Introduction

In this report, we present a systematic review on the effect of interventions that restrict the use of one or more antibiotics in food animals on the prevalence of antibiotic resistant bacteria in food animals and humans. This review has been commissioned to inform the development of a proposed WHO Guideline on the use of antibiotics in food animals, which may then serve to inform policy makers and regulatory officials on this important issue *(1)*.

Antimicrobial agents are commonly used in livestock agriculture and aquaculture for therapeutic and non-therapeutic indications *(2-4)*. These include treatment of active infections, prevention of infections in the absence of active disease, and for growth promotion. Though antimicrobial agents include drugs to treat viral, fungal, bacterial, and parasitic diseases, we have been asked to focus on antibiotics, or antibacterial drugs, for the purposes of this review.

As we gain more insight into the complexity of antibiotic resistance, there is recognition that anthropogenic activities and the wide use of antibiotics in human medicine and animal agriculture have contributed to the growing genetic pool of resistance genes found in bacteria isolated from humans, animals, and the environment *(5-8)*. There is a growing concern that the routine use of antibiotics in food animals provides strong evolutionary pressure for potentially zoonotic bacteria to develop resistance to antibiotics commonly used to treat humans *(2, 9, 10)*. Pathogenic (e.g., *Salmonella* spp., *Campylobacter* spp.) and commensal (e.g., *Escherichia coli*, *Enterococcus* spp.) bacteria, including those carrying resistance genes, can be transmitted from animals to humans through food, as well as by direct contact with animals, or through environmental sources such as contaminated water. As a result, national and international antimicrobial resistance (AMR) monitoring programs have been implemented to assess and monitor the extent of this problem *(11-14)*. Many countries have also enforced a ban on the use of specified antimicrobial agents in the feed of food animals *(15)*.

Recognizing that the use of antibiotics in food animals may lead to broader public health consequences, the WHO created the list of Critically Important Antimicrobials for human medicine (CIA) *(10, 16)*. This list classifies antimicrobials by level of importance in human medicine; it serves to prioritize the preservation of the effectiveness of antibiotics that are most important in the treatment of human infections. There is a need for recommendations on the use and restrictions of antibiotics in food animals, which specifically considers the antibiotics in the WHO CIA list. This forms the basis of the proposed WHO Guideline, the development of which may be informed by this systematic review.

There is consensus that the use of antibiotics is prevalent in food animals and that this leads to the development of antibiotic resistance in these animals. Furthermore, food animals are a source of antibiotic resistant bacteria for humans, which may then cause significant disease, morbidity, and mortality. There is, however, not consensus regarding the level of impact that antibiotic use in food animals has on antibiotic resistance in the general human population. The issue of antibiotic resistance is highly complex with a number of different biological drivers involved in the selection and persistence of antibiotic resistance genes both in the natural environment and in the presence of antibiotic use *(5, 17)*. In this systematic review and meta-analysis, we have been asked to address the following two research questions, as articulated by the WHO terms of reference *(1)*:

a) Research question one (PICOD 1)

For food animal populations of any age in any setting, does a restriction compared to not having that restriction of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in food animal populations?

b) Research question two (PICOD 2)

For human populations of any age in any setting, does a restriction compared to not having that restriction of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in human populations?

Methods

a) Search strategy

We performed this systematic review and meta-analysis using a predetermined protocol with strategic input from members of the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) and in accordance with published guidelines for reporting in systematic reviews and meta-analyses *(18)*. We identified all potentially relevant articles regardless of publication language by searching the following electronic databases:

- Agricola – Ebsco Platform (1970-present)
- AGRIS (http://agris.fao.org)
- BIOSIS Previews – Web of Knowledge Platform (1980-present)
- CAB Abstracts – Ebsco Platform (1910-present)
- MEDLINE – Ovid Platform (Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1946-present)
- EMBASE – Ovid Platform (1974-present)
- Global Index Medicus (http://www.globalhealthlibrary.net/): The non-MEDLINE indices included: AIM (AFRO), LILACS (AMRO/PAHO), IMEMR (EMRO), IMSEAR (SEARO), WPRIM (WPRO), WHOLIS (KMS), and SciELO.
- ProQuest Dissertations – ProQuest Platform
- Science Citation Index – Web of Knowledge Platform (1899-present)

A research librarian with expertise in the aforementioned databases (HG) developed the search strategy and derived three comprehensive search themes related to the populations of interest (theme 1), antimicrobial agents and drug/antimicrobial resistance (theme 2), and relevant interventions (theme 3). These three comprehensive search themes were comprised of both controlled vocabulary, such as the National Library of Medicine's MeSH (Medical Subject Heading), and keywords and were then combined using the Boolean operator "and" in varying combinations. Antibiotic-related keywords for theme 2 were derived from the WHO list of Critically Important Antimicrobials for Human Medicine *(19)* and the World Organisation for Animal Health (OIE) List of Antimicrobial Agents of Veterinary Importance *(20)*. The search strategy was peer-reviewed by a research librarian within the University of Calgary and was reviewed for content by a WHO librarian (Tomas Allen). Appendix 1 outlines the full Medline search strategy, which was modified as appropriate for other databases.

The electronic database search was enhanced by scanning bibliographies of relevant review articles and articles included into this systematic review that were published between 2010-2016, as well as by reviewing conference proceedings from major scientific meetings. Grey literature (as defined by literature produced on all levels of government, academics, business, and industry in print and electronic formats, but not controlled by commercial publishers) was identified by searching websites of relevant health agencies, professional associations, and other specialized databases. Google and Google Scholar and other internet search engines were searched for additional information. See Appendix 2 for a comprehensive list of sources and a detailed outline of the search strategy used for the grey literature. Finally, experts in the field were contacted regarding missed, ongoing, or unpublished studies.

b) Screening of abstracts for eligibility

Two individuals (KT and NC) independently reviewed all identified abstracts for eligibility using predefined criteria. Specifically, all abstracts that (a) reported on original research, (b) described an intervention that aimed to limit antibiotic use in animals, and (c) described antibiotic resistance in animals or humans were selected for full-text review. This initial stage was intentionally liberal. We only discarded abstracts that clearly did not meet the aforementioned criteria. Disagreements were resolved by consensus or third-party consultation (SC) when consensus could not be achieved.

c) Full-text review of articles

The same reviewers then performed a full-text review of articles that met the inclusion criteria. Both published and unpublished studies were eligible for inclusion. Articles were retained if they met the following PICOD criteria for research questions 1 and 2:

Populations: 1. Food-producing animals of any age falling under any of the following classifications: avian, swine, bovine, caprine, camel, equine, rabbit, ovine, fish, bees, molluscs, and crustaceans
2. Humans of any age

Intervention: A restriction of use of one or more antibiotics in food-producing animals.

Any level of restriction was considered, including complete cessation of the use of one or more antibiotics. All types of restrictions were considered, whether externally imposed by regulatory authorities or governments, or voluntary at the farm or industry level. Examples of restrictions that were considered included: any prohibition on the use of antibiotics, such as but not limited to prohibition of use for specific indications (e.g., for growth promotion or prophylaxis of disease), requirement of a prescription by a veterinarian for the use of antibiotics, organic interventions, or voluntary restrictions on farms. Additional limitations not listed above (e.g., changes to guidelines regarding preferential antibiotic use, enhanced surveillance and reporting, enhanced stewardship, innovation through research and development, and incentives for reduced antibiotic use) were considered eligible for inclusion if there was demonstrated reduction in antibiotic use.

Control/Comparator: Not having a restriction on the use of antibiotics in food animals.

Outcome: 1. The presence of antibiotic resistant bacteria and/or antibiotic resistant genetic elements and/or changes to antibiotic susceptibility (i.e. minimum inhibitory concentrations) in food-producing animal populations. All bacterial species from all possible sample types (e.g., faeces, cloacal swabs, caecal swabs, meat or carcasses, milk, eggs, nasal swabs, etc.) were considered.
2. The presence of antibiotic resistant bacteria and/or antibiotic resistant genetic elements and/or changes to antibiotic susceptibility (i.e. minimum inhibitory concentrations) in human populations. All bacterial species from all possible sample types were considered.

Design: Original studies, including both interventional and observational studies, with a comparator/control group

Studies conducted at the individual and country/area level (ecological) were considered. The comparator/control group did not have to be an external group. That is, studies with historical comparators were considered eligible for inclusion into the systematic review. Examples of included study designs were: randomized controlled trials (RCTs), non-randomized controlled trials, controlled and uncontrolled pre-post (before and after) studies, prospective cohort studies with concurrent control group, interrupted time-series and repeated measures studies, cross-sectional, and ecological studies (where there was a comparison of intervention versus comparator regions or farms). Interrater agreement for inclusion into the systematic review at the full-text review stage was good (κ=0.74).

d) Data extraction and assessment of individual study quality

Data from individual studies were extracted by KT and NC using a predesigned data extraction form. The following data elements were extracted: author, year, study design, country, species and age of animal, number of farms/animals used in analysis, sample type (e.g., faeces, solid meat, etc.), sampling point (e.g., farm, slaughter, retail), description of intervention, description of comparator, antibiotic panels, laboratory procedure, and bacteria investigated. The outcome variables of interest were the prevalence of antibiotic resistant bacteria and/or antibiotic resistant genetic elements and/or changes to antibiotic susceptibility in intervention and control groups in both animals and human subjects. Before data from all studies were extracted, the data extraction form was piloted by having the two reviewers extract data in duplicate for the first 10 individual studies (in alphabetical order). The data extracted were reviewed by two content experts on the team (SC and HB). There were no significant differences in the extracted data and no modifications to the data extraction form were required.

The same two reviewers (KT and NC) also independently assessed the methodological quality of individual studies based on pre-specified study quality indicators adapted from the Downs and Black checklist *(21)*. These items were used to assess the overall quality of reporting, external validity (generalizability), and internal validity (sources of bias) and helped inform the assessment of the global quality of the evidence (GRADE tables described below). Quality assessments were done in duplicate and any disagreements were resolved through consensus.

e) Analysis: Animal studies

Recognizing the variability in reporting with respect to study populations, interventions, outcomes of interest, and measures of association reported, we conducted four concurrent analyses for animal studies (PICOD 1).

i. *First analysis: Meta-analysis for specific bacteria and sample types*

We conducted a series of meta-analyses for all studies that reported differences in proportions as the common measure of association. Specifically, we pooled studies that reported an absolute risk difference in the prevalence of antibiotic resistance in bacteria isolated from intervention compared to control groups using DerSimonian and Laird random-effects models *(22)*. Based on this metric, a pooled negative result would indicate a lower prevalence of antibiotic resistance in the intervention group compared to the control group, whereas a positive result would indicate a higher prevalence of antibiotic resistance in the intervention group compared to the control group. A random effects model was felt necessary *a priori* regardless of actual heterogeneity of findings because the studies were known to be clinically heterogeneous, evaluating a variety of interventions across different regions globally, resulting in random effects rather than a fixed true effect across all studies. With a large number of studies, the overall power for finding statistical heterogeneity across studies was high, further highlighting the need for the use of random effects models.

Given the number of bacterial species, sample types, and antibiotic classes that could be investigated, we made an *a priori* decision to conduct pooled analyses within six bacterial/sample type groups:

1. Enterobacteriaceae in faecal samples (Enterobacteriaceae family most commonly included *Escherichia coli*, *Salmonella* spp., or unspecified Enterobacteriaceae)
2. Enterobacteriaceae in meat samples
3. *Enterococcus* spp. in faecal samples
4. *Campylobacter* spp. in faecal samples
5. *Campylobacter* spp. in meat samples
6. *Staphylococcus* spp. in milk samples

These divisions were made due to fundamental differences in these bacterial groups in terms of their microbiologic characteristics, innate antibiotic resistance, and potential for pathogenicity. Furthermore, milk, faecal, and meat samples are inherently different in terms of common bacterial species that are isolated. They are also subject to different environments and processes that may result in differing levels of baseline resistance. This was the rationale for our six separate analytic groupings. Within each of these groups, separate meta-analyses were conducted for different antibiotic classes and also for multi-drug resistance. Meta-analysis was only performed if there were six or more studies that reported risk differences in a specific antibiotic class or for multi-drug resistance, and if the studies fell within one of the six bacterial and sample type categories mentioned above. The threshold of six studies for meta-analysis was chosen because between-study variance cannot be estimated accurately with five or fewer studies, which may then lead to biased pooled estimates when meta-analysis using the DerSimonian and Laird approach is undertaken *(23)*. The quality of studies did not determine whether they were eligible for inclusion into meta-analyses; all studies that provided the necessary data to conduct meta-analysis within any one of the six bacterial and sample groupings were included. The effect of study quality on meta-analytic results was assessed using stratified analysis (see "Second analysis: Global meta-analysis section" below). The unit of analysis was at the isolate level for individual studies. When studies provided multiple point estimates (i.e. multiple antibiotics that fell within the same antibiotic class, multiple bacterial species that fell within the same genus, or multiple measures across different geographic regions), a fixed effect model with inverse variance weighting was used to generate a single point estimate per antibiotic class per study. Similarly, for longitudinal study designs with repeated measures of antibiotic resistance, the first and last data points were used to calculate risk differences within intervention and control groups.

To visually assess the pooled absolute risk difference estimates and corresponding 95% confidence intervals (CIs), forest plots were generated. Heterogeneity across studies was also evaluated using the Q statistic (significance level of $p \leq 0.10$) and the I^2 statistic *(24, 25)*. Recognizing that within and between-study variability would be substantial, we stratified our results by intervention type.

ii. Second analysis: Global meta-analysis

We performed a "global meta-analysis," which included all animal studies amenable to meta-analysis, ignoring specific bacterial species, sample types, units of analysis, and antibiotic classes, for the purposes of stratifying by quality criteria and assessing publication bias only. A single effect estimate (absolute risk difference) was generated for each study by conducting within-study meta-analysis using random effects models. This within-study meta-analysis allowed us to pool the risk difference in the prevalence of antibiotic resistance in bacteria isolated from intervention and control groups, across all tested antibiotics, all bacterial groups, and all sample types tested. We recognized that the pooled effect estimate for each study and the pooled effect estimate across all studies would not be meaningful due to the diversity of sample types, bacteria, antibiotics, and units of analysis pooled. Rather, a global meta-analysis resulted in the inclusion of more studies and thus greater power for stratified analysis by quality criteria. This stratified analysis assessed whether there was a significant difference in the pooled effect estimates in studies that met quality criteria (higher quality studies) compared to those that did not meet quality criteria (lower quality studies). Stratification was performed for three study quality criteria felt to be of highest importance:

1. Were animals included in the intervention and control group recruited from the same source population?
2. Were animals included in the intervention and control group recruited over the same period of time?
3. Was there adequate adjustment for important confounders in the analysis?

In addition, stratified analysis was performed for studies published in peer-reviewed journals versus non peer-reviewed articles (including abstracts and reports). Meta-regression was used to determine if the above four factors were significant predictors of the underlying heterogeneity.

Finally, global meta-analysis allowed assessment of publication bias as it provided a "big picture" view of all current studies examining changes to antibiotic resistance in food animals when antibiotic use is reduced. The concern about publication bias is not whether there is publication bias in each of the six specific groups of samples and bacteria that we analyzed, but rather whether there is publication bias in this entire research area, irrespective of the bacterial type, sample type, and specific antibiotic classes that are studied. Assessment of publication bias was therefore performed using all studies amenable to meta-analysis, without differentiating into the six previously described bacterial and sample groups. Publication bias was assessed using Begg's test in addition to the visual inspection of a funnel plot *(26)*. Sensitivity analysis using the Duval and Tweedie nonparametric “trim and fill” procedure was also implemented if there was any suggestion of visual asymmetry on the funnel plots *(27)*. This method considers the possibility of hypothetical “missing” studies that might exist, imputes their risk difference, and recalculates a pooled risk difference that incorporates the hypothetical studies as though they actually existed.

iii. Third analysis: Qualitative description of study results reporting phenotypic resistance

For studies that could not be included within the formal meta-analysis outlined above (described in the section “First analysis”), a semi-quantitative analysis was completed to visualize trends in phenotypic antibiotic resistance across bacterial categories (*Campylobacter* spp., Enterobacteriaceae, *Enterococcus* spp., *Staphylococcus* spp., other) and antibiotic classes. Results from individual studies were coded as green (lower prevalence of antibiotic resistance in intervention compared to control group), yellow (no difference in prevalence of antibiotic resistance between the intervention and control group) and red (higher prevalence of antibiotic resistance in intervention compared to control group). A series of decision rules were created to reduce the results into these three categories for each study:

a) If the prevalence of resistance rates to different antibiotics within the same antibiotic class were all lower or higher in the intervention compared to the control group, the antibiotic class received a green or red mark respectively.
b) If there were discordant results within an antibiotic class (i.e. there was lower resistance in the intervention group to certain antibiotics, but higher resistance to other antibiotics in the same antibiotic class) this antibiotic class received a yellow mark.
c) If there were some antibiotics that showed no difference in results across intervention and controls, while other antibiotics within the same antibiotic class showed lower or higher resistance in the intervention compared to the control group, they received a green or red mark respectively.
d) If a study did not specify the antibiotic class and simply reported on overall antibiotic resistance, they were color coded according to this overall result across all antibiotic classes and marked with an asterisk.

All decisions were corroborated by author conclusions and presence of reported statistical testing (p-values and/or 95% CIs) to determine if there was statistically significant differences between intervention and control groups.

iv. Fourth analysis: Qualitative description of study results reporting genotypic resistance

Finally, studies that reported on genetic elements, as they related to antibiotic resistance, were analyzed separately. Studies that reported on virulent genetic elements were not included within this analysis. Results were presented in two separate tables. The first summarized individual studies with respect to intervention type, population, bacteria investigated, and genes screened. A subsequent table was created to outline the numbers of studies that reported higher, lower, or no difference in prevalence of resistant genetic elements between intervention and control groups. The proportion of isolates with resistant genetic elements in the intervention versus the control group was extracted from each study. Statistical testing using Fisher’s exact test (significance level of $p<0.05$) was then conducted to determine whether differences in prevalence of resistant genetic elements between the intervention and comparator groups were significant. If data were not available to conduct these statistical tests, author conclusions were used. Results were stratified by whether authors used targeted screening (where gene detection methods were applied only to resistant isolates) versus non-targeted screening (where gene detection methods were applied to all isolates).

e) Analysis: Human studies

Due to the low numbers of human studies (addressing PICOD 2) and the relative homogeneity of these studies compared to the animal studies, a single meta-analysis was conducted regardless of sample type, bacteria isolated, and antibiotics tested. Random effects models were again used for the same reasons described above.

The unit of analysis was the sample rather than the isolate, as most human studies reported sample-level data. Stratified meta-analysis was performed by population type (individuals with direct contact to food animals such as farm workers, versus individuals without direct contact to such animals), to explore whether the associations between restriction in antibiotic use in animals and reduction in antibiotic resistance in humans were limited to specific human subpopulations. Publication bias was assessed using the same method as for animal studies, with Begg's test in addition to the visual inspection of the funnel plot *(26)*. For the studies that could not be meta-analyzed due to insufficient data, a narrative synthesis of each study was also included. A qualitative description of study results reporting genotypic resistance data was included within the same tables as the animal studies (see above "Fourth analysis: Qualitative description of study results reporting genotypic resistance").

f) GRADE tables

Judgments around the global quality of evidence required assessments of the validity of the results of individual studies for the outcomes of interest. Explicit criteria were used in making these judgments. Specifically, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group has developed a standardized and transparent methodology for assessing the global quality of evidence *(28)*. This approach has been adopted by a number of agencies and decision-making groups, including the WHO.

The quality of the outcome measures was assessed by using a standard GRADE approach as described by Guyatt et al. *(29)*. The GRADE evidence tables for the primary outcome (absolute risk difference in the prevalence of antibiotic resistance in bacteria isolated from intervention compared to control groups) were prepared for animal and human studies separately. For animal studies, although a number of stratifications was available for presentation, for brevity we limited our strata to those identified as highest priority/critically important antimicrobials as outlined by the WHO *(30)*. As described in GRADE methodology, evidence derived from RCTs starts as high quality evidence and observational studies as low quality evidence supporting estimate of intervention effects (Table 1).

Table 1: Quality assessment criteria adapted from Guyatt (2011) *(29)*

Study Design	Quality of Evidence	Lower if	Higher if
Randomized trial	High	**Risk of bias** -1 Serious -2 Very serious **Inconsistency** -1 Serious -2 very serious **Indirectness** -1 serious -2 very serious **Impression** -1 serious -2 Very serious **Publication bias** -1 Likely -2 Very likely	**Large effect** +1 Large +2 Very large **Dose response** +1 Evidence of a gradient **All plausible confounding** +1 Would reduce a demonstrated effect or +1 Would suggest a spurious effect when results show no effect
	Moderate		
Observational study	Low		
	Very low		

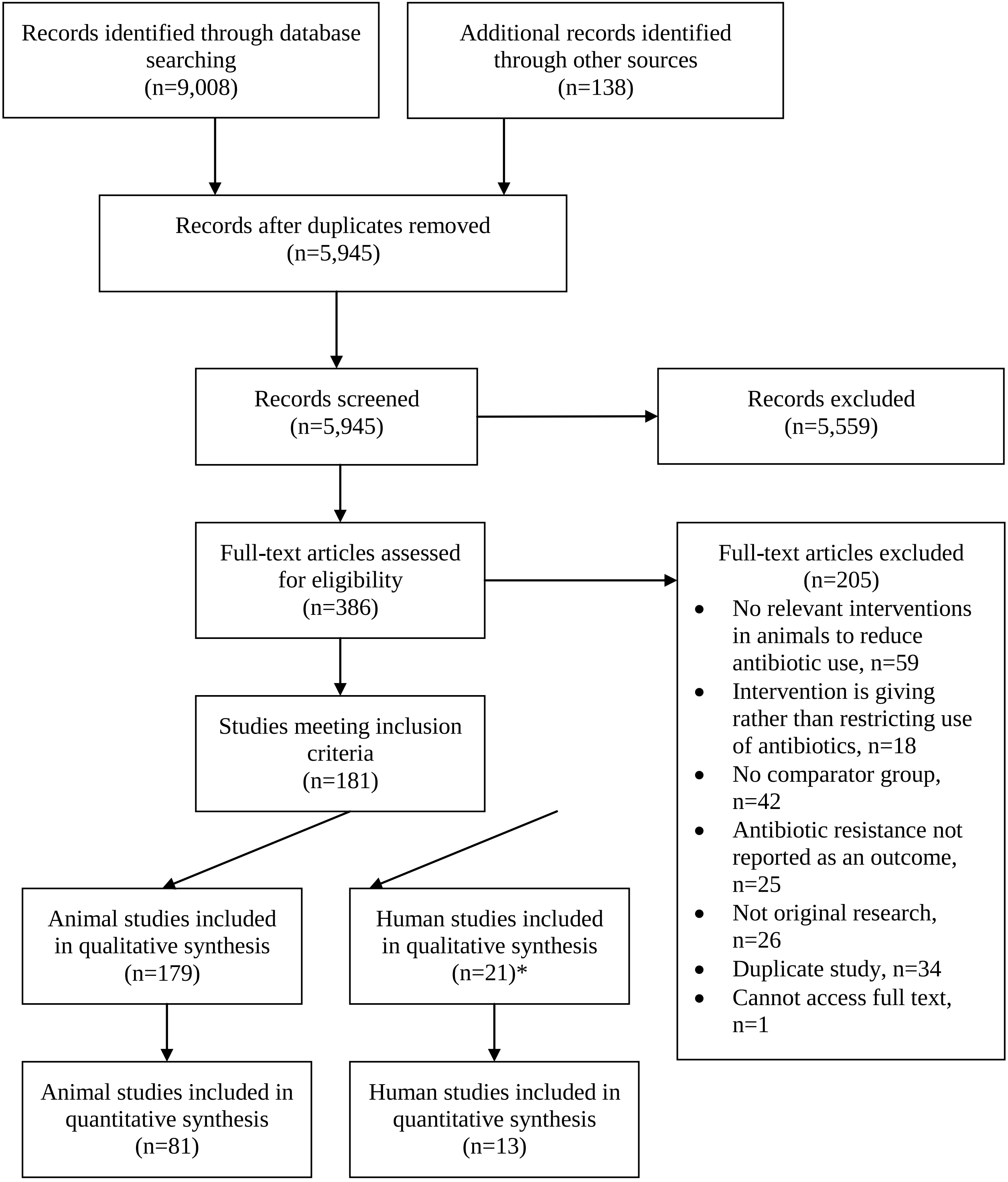
Five factors were assessed to potentially downgrade the evidence (risk of bias, inconsistency, indirectness, imprecision, and publication bias), while three factors were assessed to potentially upgrade the level of evidence (large effect, dose response, all plausible confounders or biases would result in an underestimate of the effect size). Overall, the quality of evidence for the primary outcome could fall into one of four categories including very low, low, moderate, and high. Using a consensus approach, all investigators were involved in the judgement of upgrading or downgrading the level of evidence and providing detailed reasons for doing so in the GRADE tables and accompanying notes.

Results

a) Identification of studies

The initial search strategy identified a total of 9,008 citations. An additional 56 were identified by contacting experts in the field of antibiotic use and resistance, and another 82 were identified through searching the reference lists of studies that were included in this systematic review. From these, 3,201 duplicates were removed, and 5,945 records were screened for eligibility through title and abstract review. After removal of the 5,559 records that were not relevant to either of the two research questions, 386 full-text articles were reviewed. Of these, a total of 181 studies addressed either PICOD 1 or PICOD 2 and were therefore included in the systematic review *(15, 31-210)*. The most common reasons for exclusion of articles at the full-text review stage were the absence of interventions that aimed to reduce antibiotic use in animals and the absence of a comparator group (see Figure 1 for full PRISMA flow diagram).

Figure 1: Flow diagram of the study selection process


Records identified through database searching (n=9,008)
Additional records identified through other sources (n=138)
Records after duplicates removed (n=5,945)
Records screened (n=5,945)
Records excluded (n=5,559)
Full-text articles assessed for eligibility (n=386)
Full-text articles excluded (n=205)
No relevant interventions in animals to reduce antibiotic use, n=59
Intervention is giving rather than restricting use of antibiotics, n=18
No comparator group, n=42
Antibiotic resistance not reported as an outcome, n=25
Not original research, n=26
Duplicate study, n=34
Cannot access full text, n=1
Studies meeting inclusion criteria (n=181)
Animal studies included in qualitative synthesis (n=179)
Human studies included in qualitative synthesis (n=21)*
Animal studies included in quantitative synthesis (n=81)
Human studies included in quantitative synthesis (n=13)

* Two studies were exclusively in the human population; the other 19 studied antibiotic resistance in both humans and animals and so are counted in both “animal studies” and “human studies”

Of the 181 studies, 160 reported on the outcome of antibiotic resistance in animals only, two reported on the outcome of antibiotic resistance in humans only, and 19 included both outcomes. That is, 179 studies addressed the first research question (PICOD 1) *(15, 31-108, 110-161, 163-206-210)*, while 21 studies addressed the second research question (PICOD 2) *(51, 62, 66, 72-74, 78, 90, 98, 100, 107, 109, 111, 112, 151, 162, 179, 181, 184, 196, 197)*. The results from the animal studies and human studies are reported separately. There were 19 studies that addressed both PICOD 1 and PICOD 2 and were included in both animal and human analyses.

b) Animal studies

i. *Study characteristics*

Of the 179 animal studies, 148 were articles in peer-reviewed journals, 20 were abstracts only without accompanying full-text articles, nine were dissertations, and two were government or organization reports. Poultry was the most commonly studied animal population, followed by swine and dairy cattle (see Table 2 for a summary of study characteristics and Table 3 for detailed characteristics of individual studies).

Table 2: Summary of study characteristics for animal studies

Study characteristic			**Number of studies (N=179)**
Type of article	Journal article		148
	Abstract only		20
	Dissertation		9
	Government or organization report		2
Population studied[a]	Beef cattle		20
	Dairy cattle		36
	Poultry: broilers, turkeys		87
	Poultry: egg layers		10
	Swine		61
	Goats		1
	Salmon		1
Intervention studied[a]	Externally imposed bans and reductions		36
	Organic interventions		87
		North America	44
		Europe	33
		Australia and New Zealand	4
		Asia	3
		South America	1
		Unknown	3
	Self-reported antibiotic free or related labels		38
	Voluntary reduction or withdrawal in antibiotic use		29
Sample studied[a]	Faecal/cloacal swabs/caecum		106
	Meat or carcass		53
	Milk		20
	Eggs		7
	Nasal swabs		11
	Unknown		4
Bacteria studied[a]	*Campylobacter* spp.		30
	Enterococcus spp.		39
	Enterobacteriaceae	*Escherichia coli*	58
		Salmonella spp.	31

	Yersinia enterolitica	1
	Unspecified Enterobacteriaceae	2
Staphylococcus spp.		31
Other	*Listeria monocytogenes*	3
	Lactobacillus spp.	1
	Chlamydia suis	1
	Unspecified	7

[a] Categories are not mutually exclusive and studies may be included in more than one category

Table 3: Study characteristics of individual animal studies

First author (Year)	Country	Study design	Intervention: External ban	Intervention: Organic	Intervention: Antibiotic free	Intervention: Voluntary reduction	Population sampled (Sample type)	Sample point	Unit of analysis (n)	Bacteria studied: Enterobacteriaceae	Bacteria studied: *Enterococcus* spp.	Bacteria studied: *Campylobacter* spp.	Bacteria studied: *Staphylococcus* spp.	Bacteria studied: Other	Genotypic or phenotypic resistance
Aarestrup (1995)*	Denmark	Cross-sectional			●		Broilers, egg layers (Faeces)	Farm	Isolate (29)		●				Both
Aarestrup (2000a)	Denmark	Cross-sectional				●	Broilers (Faeces)	Farm	Isolate (192)		●				Phenotypic
Aarestrup (2000b) *	Denmark, Finland, Norway	Cross-sectional	●				Broilers, pigs (Faeces)	Farm	Isolate (322)		●				Both
Aarestrup (2001)	Denmark	Longitudinal	●			●	Broilers, pigs (Caecal, cloacal)	Farm	Isolate (2,617)		●				Phenotypic
Aarestrup (2002)*	Denmark, Spain, Sweden	Cross-sectional	●				Pigs (Faeces)	Slaughter	Isolate (380)		●				Both
Abdalrahman (2015)*	USA	Cross-sectional		●			Broilers, turkeys (Meat)	Retail	Isolate (168)				●		Both
Agersø (2013)*	Denmark	Cross-sectional	●				Pigs (Caecal, faeces)	Farm, retail, slaughter	Sample (1,970)	●					Genotypic
Agga (2015)	USA	Cross-sectional			●		Beef cows (Faeces)	NR	Animal (369)	●	●				Phenotypic
Alali (2010)	USA	Cross-sectional		●			Broilers (Environment, faeces)	Farm	Isolate (70)	●					Phenotypic
Álvarez-Fernández (2012)	Spain	Cross-sectional		●			Broilers, quail (Eggs)	Retail	Isolate (120)	●					Phenotypic
Álvarez-Fernández (2013)	Spain	Cross-sectional		●			Broilers, turkey, quail (Meat)	Retail	Isolate (60)	●					Phenotypic
Avrain (2003)	France	Longitudinal			●		Broilers (Caecal)	Slaughter	Isolate (346)			●			Phenotypic
Bager (1999)	Denmark	Cross-sectional	●				Broilers, pigs (Caecal, cloacal)	Slaughter	Isolate (437)		●				Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Barlow (2008)*	Australia	Cross-sectional		•			Beef cows (Faeces, hide, meat)	Farm, slaughter	Isolate (556)					•	Genotypic
Barlow (2009)*	Australia	Cross-sectional		•			Beef cows (Faeces)	Farm, slaughter	Isolate (129)	•				•	Genotypic
Bauer-Garland (2006)	USA	Non-randomized trial				•	Broilers (Caecal)	Farm	Isolate (40)	•					Phenotypic
Bengtsson (2006)	Denmark, Norway, Sweden, The Netherlands	Longitudinal	•				Broilers, pigs (Faeces, meat)	NR	Isolate (3,473)	•	•				Phenotypic
Bennedsgaard (2006)	Denmark	Cross-sectional		•			Dairy cows (Milk)	Farm	Animal (2,311), herd (57)				•		Phenotypic
Boerlin (2001)*	Switzerland	Longitudinal	•				Pigs (Faeces)	Farm	Isolate (155)		•				Both
Bombyk (2007)*	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (NR)				•		Genotypic
Bombyk (2008)	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (405)				•		Phenotypic
Borgen (2000)*	Norway	Cross-sectional	•				Broilers (Bird, faeces)	Farm	Farm (147)		•				Both
Borgen (2001)*	Norway	Cross-sectional	•				Poultry (Carcass)	Slaughter	Isolate (150)		•				Both
Boutet (2005)	Belgium	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (1,002)				•		Phenotypic
Boyer (2012)*	USA	Cross-sectional				•	Dairy cows (Faeces)	Farm	Sample (455)	•					Both
Bunner (2007)	USA	Cross-sectional			•		Pigs (Faeces)	Farm	Isolate (1,381)	•					Phenotypic

Table 3 (continued)

			Intervention							**Bacteria studied**					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Buntenkoetter (2014)	Germany	Cross-sectional, longitudinal		•			Pigs (Environment)	Farm	Isolate (273)				•		Phenotypic
Butaye (1999)*	Belgium	Cross-sectional	•				Broilers, egg layers, pigs (Faeces)	Farm	Animal (420)		•				Both
Cho (2006)	USA	Cross-sectional		•			Dairy cows (Faeces, milk filters)	Farm, county fairs	Isolate (40)	•					Phenotypic
Cho (2007)	USA	Cross-sectional		•			Dairy cows (Faeces)	Farm, county fairs	Isolate (83)	•					Phenotypic
Cicconi-Hogan (2014)*	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (12)				•		Both
CIPARS (2016)	Canada	Longitudinal				•	Chicken (Faeces, meat)	Farm, retail, slaughter	NR	•					Phenotypic
Coalition for Animal Health (NR)	Denmark	Longitudinal	•				Pigs, poultry (Meat)	NR	NR	•	•	•			Phenotypic
Cohen Stuart (2012)*	The Netherlands	Cross-sectional		•			Broilers (Meat)	Retail	Sample (98)	•					Both
Cui (2004)	USA	Cross-sectional		•			Broilers (meat)	Retail	Sample (259)	•					Phenotypic
Cui (2005)	USA	Cross-sectional		•			Broilers (meat)	Retail	Isolate (185)	•		•			Phenotypic
Cuny (2012)	Germany	Cross-sectional			•		Pigs (Nasal swab)	Farm	Farm (82)				•		Phenotypic
Del Grosso (2000)*	Italy	Longitudinal	•				Pigs, poultry (Faeces, meat)	Retail	Isolate (1,324)		•				Both

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Desmonts (2004)	France	Longitudinal	•				Broilers (Caecal, skin)	Farm, slaughter, retail	Isolate (661)			•			Phenotypic
Docic (2003)	Hungary, Romania, Serbia	Cross-sectional				•	Pigs (Rectal swab)	Farm	Herd (39)	•					Phenotypic
Dolejska (2011)*	Czech Republic	Cross-sectional				•	Dairy cows (Milk filter, rectal swab)	Farm	Sample (463)	•					Genotypic
Dorado-García (2013)	The Netherlands	Longitudinal				•	Pigs (Nasal swab)	Farm	Farm (40)				•		Phenotypic
Dorado-García (2015a)	The Netherlands	Longitudinal				•	Pigs (Nasal swab)	Farm	Farm (36)				•		Phenotypic
Dorado-García (2015b)	The Netherlands	Longitudinal				•	Veal (Environment, nasal swab)	Farm	Farm (51)				•		Phenotypic
Dorado-García (2016)	The Netherlands	Longitudinal	•				Broilers, Pigs, Veal Calves, Dairy cows (MARAN)	Farm, slaughter	Isolate (MARAN)	•					Phenotypic
Dutil (2010)	Canada	Longitudinal				•	Broilers (Meat)	Retail	Isolate (950)	•					Phenotypic
El-Shibiny (2005)	UK	Longitudinal		•			Broilers (Faeces)	Farm	Isolate (55)			•			Phenotypic
Emborg (2002)	Denmark	Longitudinal	•				Broilers (Cloacal swab, meat)	Retail, slaughter	Isolate (DANMAP)		•				Phenotypic
Fraqueza (2014)	Portugal	Cross-sectional		•			Broilers (Caecal, meat)	Slaughter	Isolate (167)			•			Phenotypic
Gallay (2007)	France	Longitudinal	•				Broilers, pigs (Caecal)	Slaughter	Isolate (1,789)			•			Phenotypic
Garcia-Migura (2005)*	UK	Longitudinal		•			Broilers, pigs (Environment, faeces)	Farm	Farm (47)		•				Both
Garmo (2010)	Norway	Longitudinal		•			Dairy cows (Milk)	Farm	Isolate (4,209)				•		Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Ge (2004)	USA	Cross-sectional		•			Chickens (Meat)	Retail	Isolate (NR)			•			Phenotypic
Gebreyes (2006)	USA	Cross-sectional			•		Pigs (Carcass swab, faeces)	Farm, slaughter	Isolate (703)	•					Phenotypic
Gellin (1989)	USA	Cross-sectional			•	•	Pigs (Rectal swab)	Farm	Isolate (1,324)	•					Phenotypic
Gerzova (2015)*	Denmark, France, Italy, Sweden	Cross-sectional		•			Pigs (Faeces)	Slaughter	Sample (468)					•	Genotypic
Guarddon (2014)*	Spain	Cross-sectional		•			Beef cows, broilers, pigs (Meat)	Retail	Sample (200)	•					Genotypic
Halbert (2006a)*	USA	Longitudinal		•			Dairy cows (Faeces)	Farm	Isolate (1,216)			•			Both
Halbert (2006b)	USA	Longitudinal		•			Dairy cows (Environment, faeces, milk)	Farm	Isolate (2,030)			•			Phenotypic
Hammerum (2007)	Denmark	Longitudinal	•				Broilers, pigs (Faeces, meat)	Slaughter	NR		•				Phenotypic
Han (2009)	USA	Longitudinal		•			Broilers (Meat)	Retail	Isolate (165)			•			Phenotypic
Harper (2009)	USA	Cross-sectional		•			Pigs (Nasal swab)	Farm	Sample (312)				•		Phenotypic
Harvey (2009)*	USA	Cross-sectional		•			Beef cows (Faeces)	Farm	Sample (122)	NR					Genotypic
Hässig (2014)	Switzerland	Longitudinal	•				Beef cows (Faeces, nasal swab)	Farm	Farm (1,847)	•	•		•	•	Phenotypic
Heuer (2001)	Denmark	Longitudinal		•			Broilers (Cloacal)	Slaughter	Flock (160)			•			Phenotypic
Heuer (2002)*	Denmark	Longitudinal	•	•			Broilers (Cloacal)	Slaughter	Flock (162)		•				Both
Hiki (2015)*	Japan	Longitudinal				•	Broilers (Faeces)	Farm	Isolate (693)	•					Both

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Hiroi (2012)*	Japan	Cross-sectional			●		Broilers (Faeces)	Farm	Bird (32)	●					Genotypic
Hoogenboom (2008)	The Netherlands	Cross-sectional		●			Broilers, cattle, laying hens, pigs (carcass, eggs, faeces)	Farm, slaughter	Not specified (MARAN)	●	●	●			Phenotypic
Huijbers (2015)*	The Netherlands	Longitudinal		●			Broilers (Cloacal, environmental, throat swab)	Farm	Isolate (49)	●			●		Both
Jensen (2014)	Denmark	Longitudinal	●				Pigs (Faeces)	DANMAP	Isolate (DANMAP)	●					Phenotypic
Johnson (2007)	USA	Cross-sectional			●		Chicken, turkeys (Meat)	Retail, slaughter	Isolate (401)	●					Phenotypic
Johnston (2002)	USA	Cross-sectional		●			Dairy cows (Faeces)	Farm	Isolate (180)	NR					Phenotypic
Joseph (2007)	USA	Cross-sectional		●	●		Poultry (Environment)	Farm	Isolate (702)		●				Phenotypic
Joseph (2008)	USA	Cross-sectional		●	●		Broilers (Environment)	Farm	Isolate (802)	●					Phenotypic
Kassem (2017)	USA	Cross-sectional		●			Laying hens (Faeces)	Farm	Isolate (248)			●			Both
Keelara (2013)	USA	Longitudinal			●		Pigs (Environment, faeces, meat, mesenteric lymph nodes)	Farm, slaughter	Isolate (1,090)	●					Phenotypic
Kerouanton (2014)	France	Cross-sectional		●			Pigs (Faeces)	Slaughter	Isolate (373)	●					Phenotypic

Table 3 (continued)

			Intervention							**Bacteria studied**					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Khachatryan (2006)	USA	Non-randomized trial				•	Dairy cows (Faeces)	Farm	Isolate (75)	•					Phenotypic
Kieke (2006)*	USA	Cross-sectional			•		Poultry (Meat)	Retail	Isolate (100)		•				Both
Kilonzo-Nthenge (2015)	USA	Cross-sectional		•			Broilers (Meat)	Retail	Isolate (343)		•				Phenotypic
Kola (2012)*	Germany	Cross-sectional		•			Broilers (Meat)	Retail	Isolate (185)	•					Both
Kruse (1999)*	Norway	Longitudinal	•				Broilers, turkey, pigs (Carcass, faeces)	Farm, slaughter	Isolate (247)		•				Both
Kühn (2005)*	Spain, Sweden, UK	Longitudinal	•				Beef cows, broilers, pigs (Environment, faeces)	Farm	Sample (2,580)		•				Both
Lam (2012)*	The Netherlands	Longitudinal	•				Dairy cows (Faeces, milk)	MARAN	Isolate (MARAN)	•	•		•		Both
Langlois (1983)	USA	Longitudinal				•	Pigs (Faeces)	Farm	Isolate (3,094)	•					Phenotypic
Langlois (1986)	USA	Longitudinal				•	Pigs (Faeces, rectal swab)	Farm	Isolate (7,343)	•					Phenotypic
Larsen (1975)	Denmark	Longitudinal	•				Pigs (Faeces)	Farm	Isolate (443)	•					Phenotypic
Lauderdale (2007)*	Taiwan	Longitudinal	•				Chicken (Faeces)	Farm	Isolate (1,988)		•				Both
Lebek (1979)	Switzerland	Cross-sectional	•				Dairy cows (Faeces)	Farm	Isolate (63)	•					Phenotypic
Lee (2013)	South Korea	Cross-sectional		•			Chicken (Eggs)	Farm	Isolate (26)	•					Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
LeJeune (2004)	USA	Cross-sectional			•		Beef cows (Meat)	Retail	Isolate (150)	•	•				Phenotypic
Lenart-Boron (2016)*	Poland	Cross-sectional		•			Broilers (Faeces)	Farm	Isolate (98)	•					Both
Lestari (2009)	USA	Cross-sectional		•			Broilers (Meat)	Retail	Isolate (126)	•					Phenotypic
Looft (2012)*	USA	Non-randomized trial				•	Pigs (Faeces)	Farm	Sequence (133,294)	•				•	Genotypic
Lou (1995)*	USA	Cross-sectional				•	Pigs (Faeces)	Farm	Isolate (2,931)	•					Genotypic
Luangtongkum (2006)	USA	Cross-sectional		•			Broilers, turkey (Faeces)	Slaughter	Isolate (694)			•			Phenotypic
Mathew (2001)	USA	Cross-sectional			•		Pigs (Faeces)	Farm	Isolate (NR)	•					Phenotypic
Mazengia (2014)	USA	Cross-sectional		•	•		Poultry (Meat)	Retail	Isolate (106)	•					Phenotypic
Meemken (2009)	Germany	Cross-sectional		•			Pigs (Nasal swab)	Farm	Animal (678)				•		Phenotypic
Mehboob (2003)*	NR	Cross-sectional		•			Pigs (Environment, faeces)	Farm	Sample (30)	NR					Genotypic
Millar (2007)	New Zealand	Cross-sectional		•			Chicken (Meat)	Retail	Animal (6)			•			Phenotypic
Millman (2013)	USA	Cross-sectional		•	•		Chicken (Meat)	Retail	Isolate (213)	•					Phenotypic
Miranda (2007)	Spain	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (180)		•				Phenotypic
Miranda (2008a)	Spain	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (180)	•					Phenotypic

Table 3 (continued)

First author (Year)	Country	Study design	Intervention: External ban	Intervention: Organic	Intervention: Antibiotic free	Intervention: Voluntary reduction	Population sampled (Sample type)	Sample point	Unit of analysis (n)	Bacteria studied: Enterobacteriaceae	Bacteria studied: *Enterococcus* spp.	Bacteria studied: *Campylobacter* spp.	Bacteria studied: *Staphylococcus* spp.	Bacteria studied: Other	Genotypic or phenotypic resistance
Miranda (2008b)	Spain	Cross-sectional		•			Pigs (Meat)	Retail	Isolate (180)	•					Phenotypic
Miranda (2008c)	Spain	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (483)	•			•	•	Phenotypic
Miranda (2009a)	Spain	Cross-sectional		•			Beef cows (Meat)	Slaughter	Isolate (180)	•			•	•	Phenotypic
Miranda (2009b)	Spain	Cross-sectional		•			Dairy cows (Cheese)	Retail	Isolate (568)	•			•	•	Phenotypic
Miranda CD (2007)	Chile	Cross-sectional				•	Salmon (Environment, fingerling)	Farm	Isolate (70)	•					Phenotypic
Mitchell (2004)	USA	Cross-sectional		•			Dairy cows (Environmental, faeces)	Farm	Isolate (1,518)	•					Phenotypic
Mollenkopf (2014)*	USA	Cross-sectional		•	•		Chicken (Meat)	Retail	Sample (231)	•		•			Both
Morley (2011)	USA	Cross-sectional			•		Beef cows (Faeces)	Farm	Isolate (8,882)	•					Phenotypic
Nannapaneni (2009)	USA	Longitudinal	•				Chicken (Meat)	Retail	Animal (744)			•			Phenotypic
Noormohamed (2014)	USA	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (149)			•			Phenotypic
Norby (2003)	USA	Cross-sectional			•		Pigs (Faeces)	Farm	Farm (72)			•			Phenotypic
Nugent (2001)	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (180)				•		Phenotypic
Nulsen (2008)	New Zealand	Cross-sectional		•			Pigs (Faeces)	Farm	Isolate (728)	•	•				Phenotypic
Nwankwo (2014)	Nigeria	Cross-sectional				•	Chicken (Cloacal)	Farm	Isolate (45)	•					Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Obeng (2012)*	Australia	Cross-sectional			•		Broiler (Faeces)	Farm, slaughter	Isolate (251)	•					Both
O'Brien (2012)*	USA	Cross-sectional			•		Pigs (Meat)	Retail	Isolate (256)				•		Both
O'Neill (2010)*	UK	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (161)				•		Both
Osadebe (2012)	USA	Cross-sectional				•	Pigs (Nasal swab)	Farm	Sample (263)				•		Phenotypic
Österberg (2016)	Denmark, France, Italy, Sweden	Cross-sectional		•			Pigs (Faeces)	Farm, slaughter	Isolate (590)	•					Phenotypic
Pantosti (1999)*	Italy	Longitudinal	•				Poultry (Meat)	Slaughter	Sample (605)		•				Both
Park (2012)	USA	Longitudinal		•			Dairy cows (Milk)	Farm	Isolate (257)	•			•	•	Phenotypic
Patchanee (2008)	USA	Cross-sectional				•	Pigs (Faeces)	Farm	Isolate (711)	•					Phenotypic
Peng (2016)	USA	Cross-sectional		•	•		Broilers, cows, goats, laying hens, pigs (Eggs, environment, faeces)	Farm, retail	Isolate (300)	•					Phenotypic
Pettey (2008)*	USA	Cross-sectional			•		Pigs (Environment, faeces)	Farm	Isolate (242)					•	Both
Pol (2007)	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (2,503)				•	•	Phenotypic
Price (2005)*	USA	Cross-sectional			•		Chicken (Meat)	Retail	Isolate (76)			•			Both
Price (2007)	USA	Cross-sectional			•		Chicken (Meat)	Retail	Isolate (329)			•			Phenotypic

Table 3 (continued)

First author (Year)	Country	Study design	Intervention External ban	 Organic	 Antibiotic free	 Voluntary reduction	Population sampled (Sample type)	Sample point	Unit of analysis (n)	Bacteria studied Enterobacteriaceae	 *Enterococcus* spp.	 *Campylobacter* spp.	 *Staphylococcus* spp.	 Other	Genotypic or phenotypic resistance
Ray (2006)	USA	Cross-sectional		•			Dairy cows (Environment, faeces, flank swab, milk tank and filter)	Farm	Farm (129)	•					Phenotypic
Reinstein (2009)	USA	Cross-sectional			•		Beef cows (Faeces, rectal swab)	Slaughter	Isolate (60)	•					Phenotypic
Roesch (2006)	Switzerland	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (158)				•	•	Phenotypic
Rollo (2010)	USA	Cross-sectional			•		Pigs (Faeces)	Farm	Isolate (512)			•			Phenotypic
Rossa (2013)	Brazil	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (133)	•					Phenotypic
Salaheen (2016)	USA	Cross-sectional		•	•		Broilers, chicken, cows, goats, laying hens, pigs (Eggs, environment, faeces, meat)	Farm, retail	Isolate (222)			•			Phenotypic
Sanchez (2015)	USA	Cross-sectional		•	•		Chicken (Meat)	Retail	Isolate (381)	•					Phenotypic
Sapkota (2010)	USA	Cross-sectional		•			Poultry (Environment)	Farm	Isolate (100)	•					Phenotypic
Sapkota (2011)	USA	Cross-sectional		•			Broilers (Environment, faeces)	Farm	Isolate (259)		•				Phenotypic
Sapkota (2014)	USA	Cross-sectional		•			Broilers (Environment, faeces)	Farm	Isolate (103)	•					Phenotypic

Table 3 (continued)

			Intervention							**Bacteria studied**					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Sato (2004a)	USA	Cross-sectional		•			Dairy cows (Faeces)	Farm	Isolate (332)			•			Phenotypic
Sato (2004b)	Denmark, USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (483)				•		Phenotypic
Sato (2005)	USA	Cross-sectional		•			Dairy cows (Faeces)	Farm	Isolate (1,121)	•					Phenotypic
Schmidt (2015)	NR	Longitudinal			•		Beef cows (Faeces)	Slaughter	Sample (719)	•	•				Phenotypic
Schwaiger (2008)	Germany	Cross-sectional		•			Laying hens (Cloacal, eggs)	Farm	Isolate (910)	•		•			Phenotypic
Schwaiger (2010)	Germany	Cross-sectional		•			Laying hens (Cloacal, eggs)	Farm	Isolate (1,003)		•			•	Phenotypic
Siemon (2007)	USA	Cross-sectional			•		Broilers (Faeces)	Farm	Isolate (350)	•					Phenotypic
Sischo (2010)	NR	Cross-sectional				•	Dairy cows (Faeces)	Farm	Isolate (670)	•					Phenotypic
Skjøt-Rasmussen (2009)	Denmark	Longitudinal	•				Broilers, chicken (Faeces, meat)	Farm, Retail	Isolate (2,711)			•			Phenotypic
Smith (1981)	UK	Longitudinal	•				Broilers, pigs (Faeces)	Farm	Sample (200)	•					Phenotypic
Smith (2013)	USA	Cross-sectional			•		Pigs (Nasal swab)	Farm	Animal (1.085)				•		Phenotypic
Soonthornchaikul (2006)	UK	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (NR)			•			Phenotypic
Sørum (2004)	Norway	Cross-sectional	•				Chicken, turkeys (Faeces)	Farm	Isolate (94)		•				Phenotypic
Sørum (2006)*	Norway	Longitudinal	•				Broilers, turkeys (Faeces)	Farm	Sample (109)		•				Both
Stegeman (2006)	The Netherlands	Longitudinal	•				Broilers (Caecal)	Slaughter	Sample (833)		•				Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Struve (2010)	Denmark	Cross-sectional		•	•		Pigs (Caecal)	Slaughter	Isolate (868)	•					Phenotypic
Suriyasathaporn (2010)	Thailand	Longitudinal		•			Dairy cows (Milk)	Farm	Isolate (140)	•			•	•	Phenotypic
Tadesse (2009)	USA	Cross-sectional			•		Pigs (Faeces, meat)	Farm, slaughter	Isolate (1,429)	•		•			Phenotypic
Tamang (2015)*	South Korea	Cross-sectional		•			Pigs (Faeces)	Farm	Isolate (100)	•					Both
Teramoto (2016)*	USA	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (24)				•		Both
Thakur (2005)	USA	Cross-sectional			•		Pigs (Carcass swab, faeces)	Farm, slaughter	Isolate (1,459)			•			Phenotypic
Tikofsky (2003)	USA	Cross-sectional		•			Dairy cows (Milk)	Farm	Isolate (261)				•		Phenotypic
Tragesser (2006)*	USA	Cross-sectional				•	Dairy cows (Faeces)	Farm	Herd (18)	•					Both
Trost (2013)	NR	Cross-sectional		•			Chickens (Faeces, tracheal)	Farm	Isolate (148)	•					Phenotypic
Truszczyński (2006)	Denmark	Longitudinal				•	Beef cows, broilers, pigs (DANMAP)	Farm	Isolate (DANMAP)		•				Phenotypic
van den Bogaard (2000)	The Netherlands	Longitudinal	•				Broilers, pigs (Faeces)	Farm	Sample (548)		•				Phenotypic
van den Bogaard (2001)	The Netherlands	Cross-sectional				•	Broilers, laying hens, turkeys (Faeces)	Farn	Isolate (122)	•					Phenotypic
Veldman (2014)	The Netherlands	Longitudinal	•				Broilers, dairy cows, pigs, veal (Faeces)	Farm	Isolate (NR)	•	•	•			Phenotypic

Table 3 (continued)

			Intervention							Bacteria studied					
First author (Year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genotypic or phenotypic resistance**
Walk (2007)*	USA	Cross-sectional		•			Dairy cows (Faeces)	Farm	Isolate (678)	•					Genotypic
Wanninger (2016)	Switzerland	Cross-sectional				•	Pigs (Faeces, conjunctival sawb)	Farm	Isolate (18)					•	Both
Warnick (2015)	USA	Cross-sectional		•			Dairy cows (Environmental, faeces)	Farm	Isolate (1,518)	•					Phenotypic
Wyckoff (2012)	USA	Longitudinal		•			Dairy cows (Milk)	Farm	Isolate (904)				•		Phenotypic
Zawack (2016)	USA	Longitudinal	•				Chicken (Caecal, meat)	Slaughter, retail	Isolate (NR)	•		•			Phenotypic
Zhang (2005)*	USA	Cross-sectional		•			Chicken (Meat)	Retail	Isolate (71)					•	Both
Zhang (2010)	USA	Cross-sectional			•		Beef cows (Meat)	Retail	Isolate (NR)	•	•				Phenotypic
Zhang (2011)	USA	Cross-sectional			•		Chicken (Meat)	Retail	Isolate (329)	•	•				Phenotypic
Zwonitzer (2016)	USA	Cross-sectional		•			Pigs (Faeces)	Farm	Isolate (491)	•					Phenotypic

*Genetic data summarized in Table 13

Abbreviations: DANMAP - Danish Integrated Antimicrobial Resistance Monitoring and Research Programme; MARAN - Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands; NR - not reported; UK - United Kingdom; USA - United States of America

Interventions were classified into four categories: 1) Externally imposed bans or restrictions of antibiotic use (n=36 studies); 2) Organic interventions, as defined by the study and the country-specific regulations for organic certification (n=87 studies); 3) Self-reported and self-labeling of antibiotic-free and related interventions, such as free-range or pasture (n=38 studies); and 4) Voluntary reduction or cessation of antibiotic use (n=29 studies). These intervention categories were not mutually exclusive. For example, studies that reported antibiotic resistance in antibiotic-free versus organic versus conventional meats were considered to include both “Organic” and “Antibiotic-Free” interventions.

A variety of samples were tested for antibiotic resistance across studies. Most commonly, studies used faecal or caecal samples (n=106 studies), followed by meat or carcass samples (n=53 studies). In addition, a wide variety of bacteria were studied including *Campylobacter* spp., *Enterococcus* spp., Enterobacteriaceae family of bacteria, and *Staphylococcus* spp. See Appendix 3 for flow charts illustrating the sample points, sample types, and bacteria studied for each of the four classes of interventions.

Studies were predominantly from the United States (n=81). A large number of studies were European in origin (n=78), with many of these comparing antibiotic resistance across different European countries. The countries in Europe with greatest representation were Denmark (n=21) and Spain (n=11). Few studies originated from Asia (n=6) and only one study was from Africa. Five studies did not specify the country of origin (Figure 2).

Figure 2: Geographic representation of countries* from which animal studies (human studies in parentheses) originate, with enlarged European insert

(The global map is currently being reproduced and to be uploaded in due course)

ii. Study quality

Study quality was assessed for all 179 studies that examine antibiotic resistance in animals (Table 4).

Table 4: Assessment of study quality for individual animal studies

First author (Year)	Is hypothesis/aim/objective of the study clearly defined?	Are the characteristics of the animals/humans included in the study clearly described?	Are the interventions of interest clearly described?	Are the main outcomes clearly described in the Introduction or Methods section?	Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)	Were the subjects included in the study representative of the entire population from which they were recruited?	Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)	Were animals/humans included in the intervention and control groups recruited over the same period of time?	Was there adequate adjustment for important confounders in the analysis?
Aarestrup (1995)	Yes	No	Yes	Yes	No	Unknown	No	No	No
Aarestrup (2000a)	Yes	No	Yes	Yes	Yes	Yes	Yes	N/A	No
Aarestrup (2000b)	Yes	No	Yes	Yes	No	Unknown	No	Yes	No
Aarestrup (2001)	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Aarestrup (2002)	Yes	No	No	Yes	No	No	Unknown	No	No
Abdalrahman (2015)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Agersø (2013)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No
Agga (2015)	Yes	No	No	Yes	No	No	Unknown	Unknown	No
Alali (2010)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Álvarez-Fernández (2012)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Álvarez-Fernández (2013)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Avrain (2003)	Yes	No	Yes	Yes	No	Yes	Unknown	Yes	Unknown

Table 4 (continued)

First author (Year)	Is hypothesis/aim/objective of the study clearly defined?	Are the characteristics of the animals/humans included in the study clearly described?	Are the interventions of interest clearly described?	Are the main outcomes clearly described in the Introduction or Methods section?	Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)	Were the subjects included in the study representative of the entire population from which they were recruited?	Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)	Were animals/humans included in the intervention and control groups recruited over the same period of time?	Was there adequate adjustment for important confounders in the analysis?
Bager (1999)	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No
Barlow (2008)	Yes	No	No	Yes	No	No	No	Yes	No
Barlow (2009)	Yes	No	No	Yes	No	Yes	No	Yes	No
Bauer Garland (2006)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Bengtsson (2006)	Yes	No	Yes	Yes	No	Yes	No	Yes	No
Bennedsgaard (2006)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Boerlin (2001)	Yes	No	Yes	Yes	Yes	Unknown	Yes	Yes	No
Bombyk (2007)	Yes	No	No	Yes	No	No	No	Unknown	No
Bombyk (2008)	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes
Borgen (2000)	Yes	No	Yes	Yes	Yes	No	No	N/A	No
Borgen (2001)	Yes	No	Yes	Yes	No	Yes	No	Yes	No
Boutet (2005)	Yes	No	Yes	Yes	No	No	Yes	Yes	Unknown
Boyer (2012)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Bunner (2007)	Yes	Unknown	No	Yes	Yes	Unknown	Yes	Yes	Yes
Buntenkoetter (2014)	Yes	No	Yes	Yes	Yes	No	No	Yes	No
Butaye (1999)	No	No	No	Yes	No	No	No	No	No

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Cho (2006)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Cho (2007)	Yes	No	Yes	Yes	No	No	No	Yes	No
Cicconi-Hogan (2014)	Yes	No	No	Yes	Yes	No	Yes	Yes	No
CIPARS (2016)	Yes	No	Yes	Yes	No	Yes	Unknown	N/A	No
Coalition for Animal Health (NR)	Yes	No	Yes	Yes	No	Unknown	Unknown	N/A	No
Cohen Stuart (2012)	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
Cui (2004)	Yes	Yes	Yes	Yes	No	No	No	Yes	No
Cui (2005)	Yes	Yes	Yes	Yes	No	No	No	Yes	No
Cuny (2012)	No	Unknown	Unknown	Unknown	No	No	Yes	Unknown	No
Del Grosso (2000)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	No
Desmonts (2004)	Yes	No	Yes	Yes	No	No	Yes	Yes	No
Docic (2003)	Yes	No	Yes	Yes	No	Yes	No	No	No
Dolejska (2011)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Dorado-García (2013)	Yes	No	Yes	Yes	No	Yes	Yes	N/A	No
Dorado-García (2015a)	Yes	Yes	Yes	Yes	Yes	Unknown	Yes	N/A	Yes
Dorado-García (2015b)	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Dorado-García (2016)	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Dutil (2010)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
El-Shibiny (2005)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Unknown
Emborg (2002)	Yes	No	Yes	Yes	Yes	Yes	Yes	N/A	Unknown
Fraqueza (2014)	Yes	No	Yes	Yes	Yes	Unknown	No	Yes	No
Gallay (2007)	Yes	No	No	Yes	No	Yes	Yes	Yes	No
Garcia Migura (2005)	Yes	No	No	Yes	No	Unknown	Yes	Yes	No
Garmo (2010)	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
Ge (2004)	Yes	No	No	Yes	No	No	No	Yes	Unknown
Gebreyes (2006)	Yes	No	Yes	Yes	Yes	No	Unknown	Yes	No
Gellin (1989)	Yes	No	Yes	Yes	No	No	Unknown	Unknown	No
Gerzova (2015)	Yes	No	No	Yes	Yes	Yes	Unknown	Yes	No
Guarddon (2014)	Yes	Yes	Yes	Yes	No	Unknown	Yes	Yes	No
Halbert (2006a)	Yes	No	No	Yes	No	Unknown	No	Unknown	No
Halbert (2006b)	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No
Hammerum (2007)	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Unknown

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Han (2009)	Yes	Yes	Yes	Yes	No	No	Yes	No	No
Harper (2009)	Yes	No	No	No	No	No	Unknown	Unknown	Unknown
Harvey (2009)	Yes	Yes	Yes	Yes	No	No	Yes	Unknown	No
Hässig (2014)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No
Heuer (2001)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No
Heuer (2002)	Yes	Yes	Yes	Yes	No	Unknown	No	Yes	No
Hiki (2015)	Yes	No	Yes	Yes	No	Yes	Yes	N/A	No
Hiroi (2012)	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Hoogenboom (2008)	Yes	No	No	Yes	No	Unknown	Unknown	No	Unknown
Huijbers (2015)	Yes	Yes	No	Yes	No	Yes	No	No	No
Jensen (2014)	No	No	Yes	No	No	No	No	Yes	Unknown
Johnson (2007)	Yes	Yes	No	Yes	No	Unknown	Unknown	Unknown	Yes
Johnston (2002)	Yes	No	Yes	Yes	No	No	No	Yes	No
Joseph (2007)	Yes	No	Yes	Yes	No	No	No	Unknown	No
Joseph (2008)	Yes	No	No	Yes	No	Unknown	Unknown	Unknown	Unknown
Kassem (2017)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Keelara (2013)	Yes	No	Yes	Yes	No	Unknown	No	Yes	Yes
Kerouanton (2014)	Yes	No	No	No	No	No	Unknown	Yes	No
Khachatryan (2006)	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No
Kieke (2006)	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Kilonzo-Nthenge (2015)	Yes	Yes	No	Yes	Yes	No	No	No	No
Kola (2012)	Yes	No	No	Yes	Yes	No	No	Yes	Yes
Kruse (1999)	No	No	Yes	Yes	No	Unknown	Unknown	No	No
Kühn (2005)	Yes	No	Yes	Yes	No	Unknown	No	Yes	Unknown
Lam (2012)	Yes	No	Yes	No	No	Yes	Unknown	N/A	Unknown
Langlois (1983)	Yes	No	Yes	No	No	No	Yes	N/A	Unknown
Langlois (1986)	Yes	No	Yes	Yes	No	No	No	Yes	No
Larsen (1975)*	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Lauderdale (2007)	Yes	No	Yes	Yes	No	Yes	Yes	N/A	No
Lebek (1979)	Unknown	No	Yes	Yes	No	No	Yes	N/A	No
Lee (2013)	Yes	No	No	Yes	No	No	Unknown	Yes	No
LeJeune (2004)	Yes	No	No	Yes	No	Unknown	Unknown	Yes	No

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Lenart-Boron (2016)	Yes	No	Yes	Yes	No	No	No	Yes	No
Lestari (2009)	Yes	Yes	Yes	Yes	No	No	No	Yes	No
Looft (2012)	Yes	Yes	Yes	No	No	No	Yes	Yes	No
Lou (1995)	Yes	Yes	Yes	Yes	No	No	Yes	N/A	No
Luangtongkum (2006)	Yes	No	Yes	Yes	No	Unknown	No	Yes	No
Mathew (2001)	Yes	No	Yes	Yes	No	Yes	Unknown	Unknown	No
Mazengia (2014)	Yes	No	No	Yes	No	No	Yes	Yes	No
Meemken (2009)	Yes	No	No	No	No	Unknown	Unknown	Unknown	No
Mehboob (2003)	Yes	No	Yes	Yes	No	Unknown	Unknown	Unknown	Unknown
Millar (2007)	No	No	No	No	No	No	No	Yes	No
Millman (2013)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda (2007)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda (2008a)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda (2008b)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda (2008c)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda (2009a)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	No

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Miranda (2009b)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Miranda CD (2007)	Yes	No	Yes	Yes	Yes	No	Unknown	Yes	No
Mitchell (2004)	Yes	No	No	Yes	Yes	Unknown	Yes	Yes	Yes
Mollenkopf (2014)	Yes	Yes	No	Yes	No	No	Unknown	Yes	Yes
Morley (2011)	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Nannapaneni (2009)	Yes	No	Yes	Yes	No	Unknown	Yes	N/A	No
Noormohamed (2014)	Yes	No	No	No	No	Unknown	Unknown	Yes	No
Norby (2003)	Yes	No	Yes	Yes	No	Yes	Yes	Unknown	Unknown
Nugent (2001)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	Unknown
Nulsen (2008)	Yes	No	Yes	No	No	Unknown	Unknown	Yes	No
Nwankwo (2014)	Yes	No	Yes	Yes	No	Unknown	Unknown	Unknown	No
Obeng (2012)	Yes	No	Yes	Yes	No	No	Unknown	Yes	Unknown
O'Brien (2012)	Yes	Yes	No	Yes	Yes	Yes	Unknown	Yes	No
O'Neill (2010)	Yes	No	Yes	Yes	No	Unknown	Unknown	Unknown	No
Osadebe (2012)	Yes	Yes	Yes	Yes	No	Unknown	Yes	Yes	Yes
Österberg (2016)	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Pantosti (1999)	Yes	No	Yes	No	No	No	Yes	N/A	No
Park (2012)	Yes	No	Yes	Yes	No	Unknown	Yes	N/A	No
Patchanee (2008)	Yes	No	No	Yes	No	No	Unknown	Unknown	No
Peng (2016)	Yes	No	No	Yes	No	Unknown	No	Unknown	No
Pettey (2008)	No	No	Yes	No	No	No	Unknown	Unknown	No
Pol (2007)	Yes	No	Yes	Yes	No	No	Unknown	Unknown	No
Price (2005)	Yes	No	No	Yes	Yes	No	Unknown	Yes	No
Price (2007)	Yes	No	No	Yes	Yes	No	Unknown	Yes	No
Ray (2006)	Yes	No	Yes	Yes	Yes	Yes	Unknown	Yes	Yes
Reinstein (2009)	Yes	No	Yes	No	Yes	Unknown	Unknown	Unknown	No
Roesch (2006)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Rollo (2010)	Yes	Yes	Yes	Yes	Yes	Unknown	Yes	Yes	Yes
Rossa (2013)	Yes	Yes	Yes	Yes	No	No	No	Yes	No
Salaheen (2016)	Yes	No	No	Yes	No	Unknown	No	Unknown	No
Sanchez (2015)	Yes	No	No	Yes	No	No	No	Unknown	No
Sapkota (2010)	Yes	No	No	Yes	No	Unknown	Unknown	Unknown	Unknown

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Sapkota (2011)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Unknown
Sapkota (2014)	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Unknown
Sato (2004a)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	Yes
Sato (2004b)	Yes	No	Yes	Yes	Yes	No	Unknown	Yes	No
Sato (2005)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	Yes
Schmidt (2015)	Yes	No	No	No	No	Unknown	Unknown	Yes	Unknown
Schwaiger (2008)	Yes	No	No	Yes	No	Unknown	Unknown	Yes	No
Schwaiger (2010)	Yes	No	No	Yes	No	Unknown	Unknown	Yes	No
Siemon (2007)	Yes	Yes	Yes	Yes	No	Unknown	Unknown	Yes	No
Sischo (2010)	Yes	No	Yes	Yes	No	No	Yes	Yes	Unknown
Skjøt-Rasmussen (2009)	Yes	Yes	Yes	Yes	No	Unknown	No	N/A	No
Smith (1981)	No	No	Yes	No	No	Yes	Unknown	N/A	No
Smith (2013)	No	Yes	No	No	Yes	No	Unknown	Yes	Unknown
Soonthornchaikul (2006)	Yes	No	No	Yes	No	No	Unknown	Unknown	No
Sørum (2004)	Yes	No	No	Yes	Yes	Unknown	Unknown	Unknown	No
Sørum (2006)	Yes	No	Yes	Yes	No	No	Yes	N/A	No

Table 4 (continued)

First author (Year)	Is hypothesis/aim/objective of the study clearly defined?	Are the characteristics of the animals/humans included in the study clearly described?	Are the interventions of interest clearly described?	Are the main outcomes clearly described in the Introduction or Methods section?	Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)	Were the subjects included in the study representative of the entire population from which they were recruited?	Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)	Were animals/humans included in the intervention and control groups recruited over the same period of time?	Was there adequate adjustment for important confounders in the analysis?
Stegeman (2006)	Yes	No	Yes	Yes	Yes	Yes	Yes	N/A	Yes
Struve (2010)	Yes	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Suriyasathaporn (2010)	Yes	Yes	Yes	Yes	No	Yes	Yes	N/A	No
Tadesse (2009)	Yes	Yes	No	Yes	No	No	No	Unknown	No
Tamang (2015)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Teramoto (2016)	Yes	No	No	Yes	No	No	No	Yes	No
Thakur (2005)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	No
Tikofsky (2007)	Yes	Yes	Yes	Yes	Yes	Unknown	Yes	Yes	No
Tragesser (2006)	Yes	No	Yes	Yes	Yes	No	Yes	Unknown	No
Trost (2013)	Yes	No	Yes	Yes	No	Unknown	Unknown	Unknown	Unknown
Truszczyński (2006)	Yes	No	Yes	Yes	No	Yes	Yes	N/A	Unknown
van den Bogaard (2000)	Yes	No	Yes	Unknown	No	Unknown	Unknown	N/A	No
van den Bogaard (2001)	Yes	No	No	Yes	No	Unknown	No	Unknown	No
Veldman (2014)	No	No	No	No	No	Yes	Yes	N/A	No
Walk (2007)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Wanninger (2016)	Yes	No	No	Yes	Yes	Unknown	Unknown	Yes	No

Table 4 (continued)

First author (Year)	**Is hypothesis/aim/objective of the study clearly defined?**	**Are the characteristics of the animals/humans included in the study clearly described?**	**Are the interventions of interest clearly described?**	**Are the main outcomes clearly described in the Introduction or Methods section?**	**Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CI, etc.)**	**Were the subjects included in the study representative of the entire population from which they were recruited?**	**Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)**	**Were animals/humans included in the intervention and control groups recruited over the same period of time?**	**Was there adequate adjustment for important confounders in the analysis?**
Warnick (2015)	Yes	No	No	Yes	Yes	Unknown	Unknown	Yes	Yes
Wyckoff (2012)	Yes	No	Yes	Yes	No	Unknown	Yes	Yes	Unknown
Zawack (2016)	Yes	No	No	No	No	Unknown	Unknown	N/A	No
Zhang (2005)	Yes	No	No	Yes	No	Unknown	Unknown	Yes	No
Zhang (2010)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No
Zhang (2011)	Yes	Yes	No	Yes	No	No	Yes	Yes	No
Zwonitzer (2016)	Yes	No	No	Yes	No	Unknown	No	Yes	No

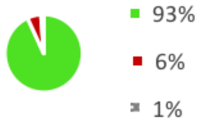
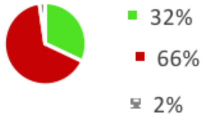
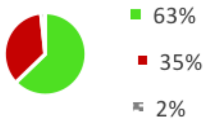
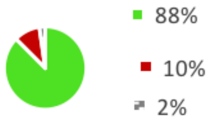
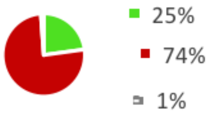
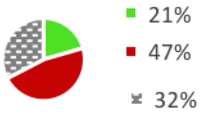
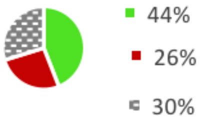
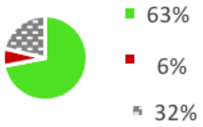
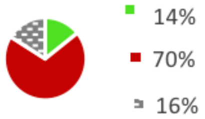
Yes - study quality criteria met; No - study quality criteria not met; Unknown - insufficient information to assess study quality

Abbreviations: N/A - not applicable, NR - not reported

*Study quality assessment incomplete - awaiting translation

Nearly all studies had a clearly defined research question or objective. Other strengths in study quality included clearly defined interventions and outcomes and that intervention and control groups were recruited over the same period of time. See Table 5 for a summary of proportions of studies meeting each study quality criterion (marked green on pie charts), proportions of studies not meeting each study quality criterion (marked red on pie charts), and proportions of studies where study quality could not be assessed based on the data reported (marked grey on pie charts).

Table 5: Proportion of studies meeting study quality criteria

Study quality criteria	Pie chart of studies meeting study quality criteria
Is hypothesis/aim/objective of the study clearly defined?	
Are the characteristics of the animals/humans included in the study clearly described?	
Are the interventions of interest clearly described?	
Are the main outcomes clearly described in the Introduction or Methods section?	
Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CIs, etc.)	
Were the subjects included in the study representative of the entire population from which they were recruited?	
Were animals/humans included in the intervention and control groups recruited from the same source population? (i.e. Were they sampled from the same population?)	
Were animals/humans included in the intervention and control groups recruited over the same period of time?	
Was there adequate adjustment for important confounders in the analysis?	

🟩Green: Proportion of studies meeting study quality criteria; 🟥Red: Proportion of studies not meeting study quality criteria; ▨Grey: Proportion of studies where there was insufficient information to assess study quality criteria

An area of weaker study quality was the general lack of description of the study populations and management practices on farms and slaughterhouses. Without a clear description of sample characteristics, it was often difficult to assess whether intervention and control groups were comparable and whether they were representative of the source population. There also tended to be a lack of description of the interventions that aimed to reduce antibiotic use in animals, specifically in studies that sampled at the retail level (e.g., studies that compared antibiotic resistance in retail meats labeled as organic or antibiotic-free or other related labels versus conventional meats). For labels without rigorous requirements or certification such as for pasture-raised, free-range, or raised without antibiotics, the label itself, without a clear description of the underlying farm management practices, provides no detail regarding co-interventions and degree of antibiotic use, such as whether antibiotics are allowed therapeutically. For studies with organic interventions, many studies again tended to use the term "organic" to describe the intervention without any other detail, and despite rigorous processes for certification, this label alone is an inadequate description of the intervention, given that organic requirements are generally country or region-specific. Furthermore, few studies adjusted for potential confounders, despite the many variables that are likely to confound the complex association between antibiotic resistance and antibiotic use in animals. To address these study quality limitations, we conducted stratified analysis based on study quality criteria to determine whether there were any differences in pooled effect estimates for higher versus lower quality studies (see section "Stratified analysis: study quality criteria").

iii. Synthesis of results

Of the 179 studies reporting antibiotic resistance in animals, 81 were included in meta-analyses. Because of the heterogeneity of the studies, with different bacteria, sample types (faeces versus carcass versus milk samples), and sample points (farm versus slaughter versus retail), we chose to conduct a series of meta-analyses rather than pooling all studies simultaneously in a single meta-analysis. We pooled the absolute risk differences in antibiotic resistance for individual antibiotic classes for: 1) Enterobacteriaceae in faecal samples; 2) Enterobacteriaceae in meat samples; 3) *Enterococcus* spp. in faecal samples; 4) *Campylobacter* spp. in faecal samples; 5) *Campylobacter* spp. in meat samples; and 6) *Staphylococcus* spp. in milk samples.

1. Enterobacteriaceae in faecal samples

The Enterobacteriaceae are a large family of bacteria, though all studies that were meta-analyzed reported antibiotic resistance for only three groups: 1) *E. coli*, 2) *Salmonella* spp., and 3) non-specified faecal coliforms. The most commonly studied bacterial species was *E. coli.* Depending on the antibiotic class, absolute risk differences in antibiotic resistance in Enterobacteriaceae were pooled for 16 to 21 studies (Table 6).

Table 6: Pooled absolute risk differences of antibiotic resistance for Enterobacteriaceae isolates in faecal samples

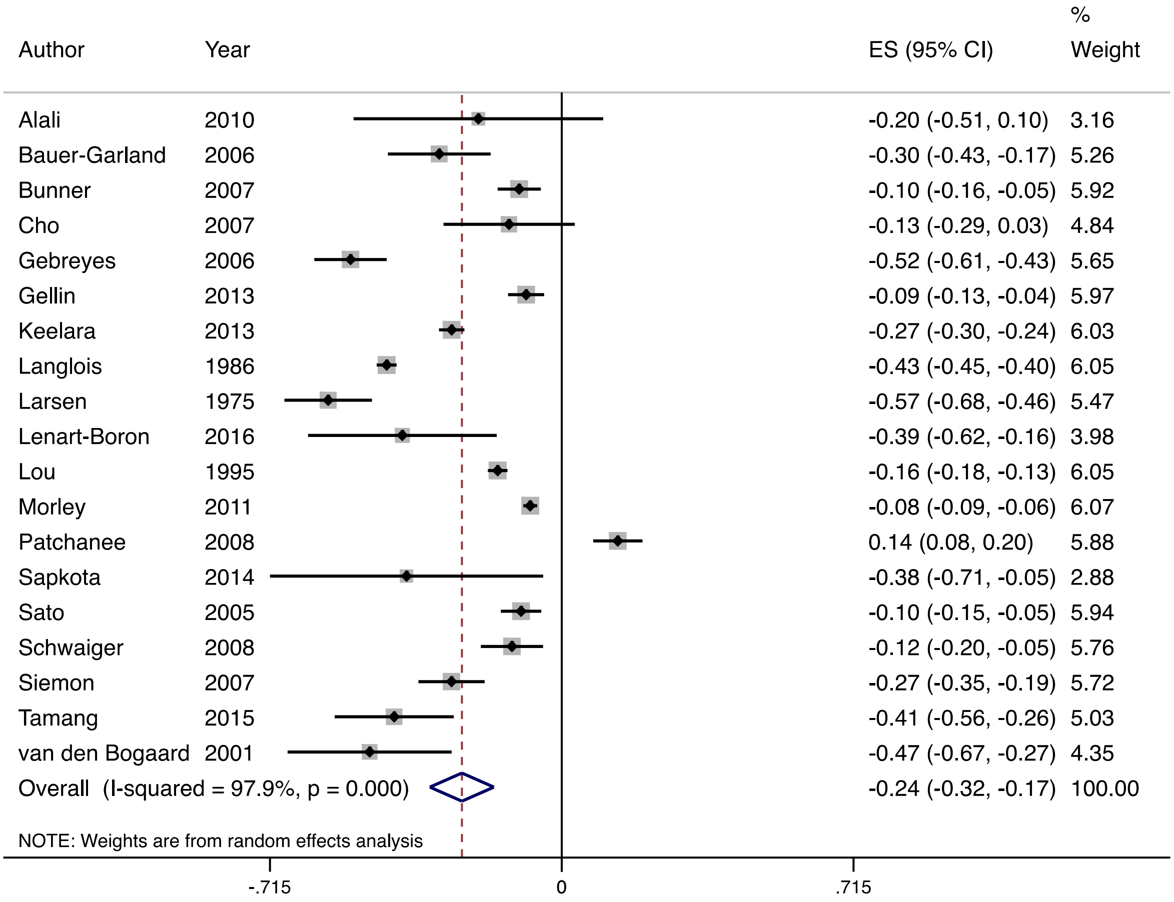
Antibiotic class	No. studies	Pooled absolute risk difference (95% CI)
Aminoglycosides	21	-0.12 (-0.17, -0.07)
Amphenicols	16	-0.04 (-0.06, -0.03)
Cephalosporins	17	-0.01 (-0.04, 0.01)
Penicillins	20	-0.12 (-0.18, -0.07)
Quinolones	17[a]	-0.01 (-0.02, -0.00)
Sulfonamides	20	-0.06 (-0.09, -0.02)
Tetracyclines	21	-0.16 (-0.27, -0.05)

[a] Two of the 17 studies were excluded from meta-analysis due to a standard error of 0, with no reported resistance in either the intervention or comparator groups.

All pooled estimates across antibiotic classes were less than zero, indicating that the pooled risk of antibiotic resistance in the intervention group was lower than that in the control group. These were statistically significant, with the upper limit of the 95% confidence interval not crossing 0, for all antibiotic classes except for cephalosporins and quinolones. The pooled absolute risk difference of antibiotic resistance was highest for tetracyclines at -0.16 (95% CI -0.27, -0.05). This corresponds to a 16% reduction in the percentage of isolates that are antibiotic resistant in the intervention group compared to the control group. The pooled absolute risk difference was lowest for cephalosporins and quinolones at -0.01 (95% CI -0.04, 0.01) and -0.01 (95% CI -0.02, -0.00) respectively, corresponding to a 1% reduction in the percentage of isolates that are antibiotic resistant in the intervention group compared to the control group for both antibiotic classes. See Appendix 4 Figures 1-7 for forest plots of absolute risk differences of antibiotic resistance for each of the seven classes of antibiotics. There was significant heterogeneity across studies, with an I^2 between 92.8% and 99.3% and Cochran Q test p-values <0.05 for all antibiotic classes for which meta-analysis was undertaken. Meta-analysis was not undertaken for the following antibiotic classes due to insufficient numbers of studies: aminocyclitols (2 studies), carbapenems (1 study), nitrofurantoins (2 studies), and polymyxins (4 studies).

A meta-analysis of 19 studies reporting multi-drug resistance to antibiotics was also conducted. The pooled absolute risk difference of multi-drug resistance was -0.24 (95% CI -0.32, -0.17). That is, the pooled proportion of isolates that were resistant to multiple antibiotics was 24% lower in intervention groups compared to control groups (Figure 3).

Figure 3: Forest plot of absolute risk differences of multi-drug resistance for Enterobacteriaceae isolates in faecal samples



2. *Enterobacteriaceae in meat samples*

Similar to faecal samples, all studies of Enterobacteriaceae in meat samples studied *E. coli*, *Salmonella* spp., or unspecified coliforms. Depending on the antibiotic class, the absolute risk differences in antibiotic resistance in Enterobacteriaceae were pooled for 11 to 13 studies (Table 7).

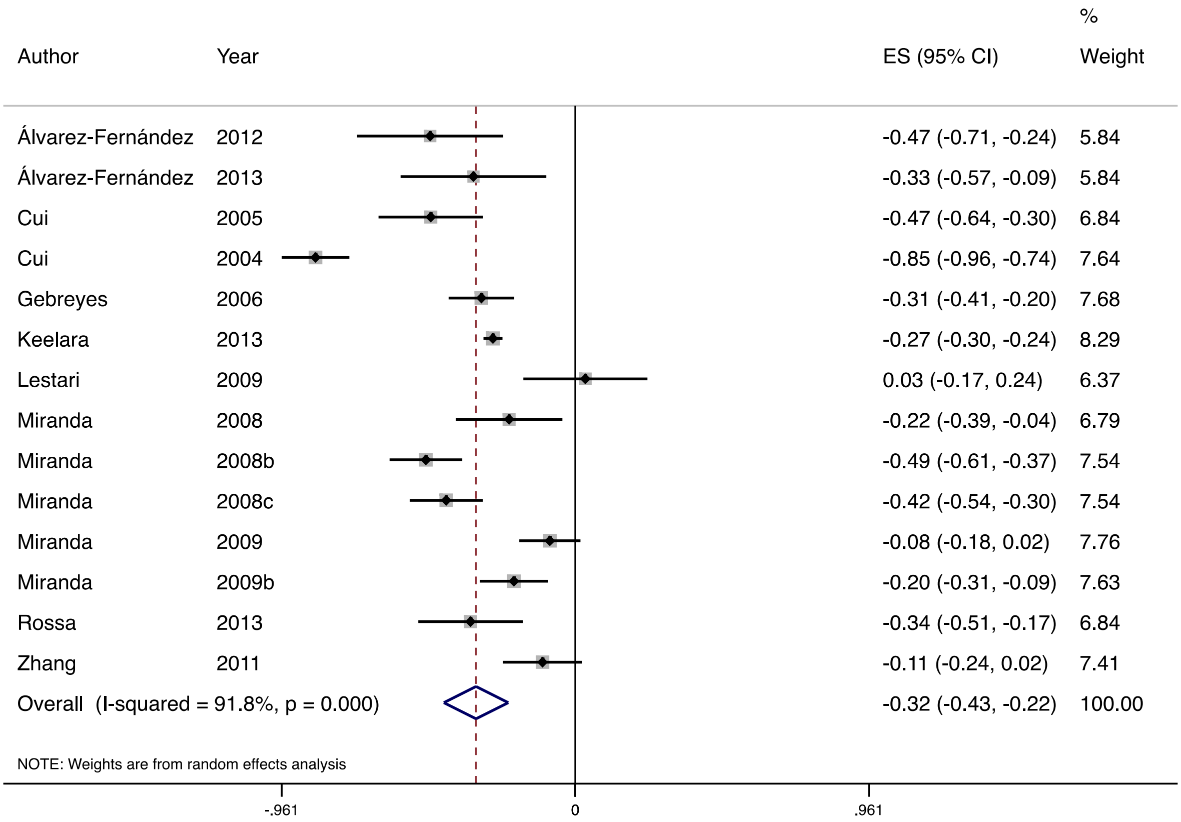
Table 7: Pooled absolute risk differences of antibiotic resistance for Enterobacteriaceae isolates in meat samples

Antibiotic class	No. studies	Pooled absolute risk difference (95% CI)
Aminoglycosides	12	-0.07 (-0.12, -0.02)
Amphenicols	11	-0.08 (-0.14, -0.03)
Cephalosporins	11	-0.07 (-0.14, 0.01)
Penicillins	11	-0.16 (-0.25, -0.08)
Quinolones	12	-0.09 (-0.17, -0.02)
Sulfonamides	13	-0.23 (-0.32, -0.13)
Tetracyclines	13	-0.20 (-0.36, -0.03)

All pooled estimates across antibiotic classes were less than zero, indicating that the pooled risk of antibiotic resistance in the intervention group was lower than that in the control group. These were statistically significant for all antibiotic classes except for cephalosporins (risk difference -0.07 [95% CI -0.14, 0.01]). The pooled absolute risk difference of antibiotic resistance was highest for sulfonamides at -0.23 (95% CI -0.32, -0.13). See Appendix 4 Figures 8-14 for forest plots of absolute risk differences of antibiotic resistance for each of the seven classes of antibiotics. There was significant heterogeneity across studies, with an I^2 between 82.3% and 97.9% and Cochran Q test p-value <0.05 for all studies for which meta-analysis was undertaken. Meta-analysis was not undertaken for the following antibiotic classes due to insufficient numbers of studies: carbapenems (1 study), cyclic esters (3 studies), macrolides (1 study), and nitrofurantoins (5 studies).

In addition, a meta-analysis of 14 studies reporting multi-drug resistance to antibiotics was conducted. The pooled absolute risk difference of multi-drug resistance was -0.32 (95% CI -0.43, -0.22). That is, the proportion of isolates that were resistant to multiple antibiotics was 32% lower in intervention groups compared to control groups (Figure 4).

Figure 4: Forest plot of absolute risk differences of multi-drug resistance for Enterobacteriaceae isolates in meat samples



3. *Enterococcus spp. in faecal samples*

For *Enterococcus* spp. in faecal samples, depending on the antibiotic class, the absolute risk differences in antibiotic resistance were pooled for 7 to 12 studies (Table 8).

Table 8: Pooled absolute risk differences of antibiotic resistance for *Enterococcus* spp. isolates in faecal samples

Antibiotic class	No. studies	Pooled absolute risk difference (95% CI)
Aminoglycosides	7	-0.13 (-0.23, -0.02)
Glycopeptides	12	-0.22 (-0.32, -0.12)
Macrolides	10	-0.39 (-0.56, -0.23)
Penicillins	7	-0.10 (-0.18, -0.02)
Streptogramins	8	-0.31 (-0.46, -0.17)
Tetracyclines	7	-0.30 (-0.48, -0.13)

Similar to the faecal and meat samples for Enterobacteriaceae, all pooled estimates across antibiotic classes were less than zero, indicating that the pooled risk of antibiotic resistance in the intervention group was lower than that in the control group. These were statistically significant for all antibiotic classes. The pooled absolute risk difference of antibiotic resistance was highest for macrolides at -0.39 (95% CI -0.56, -0.23). It was the lowest for penicillins, though the absolute risk difference was still -10% for this antibiotic class (-0.10 [95% CI -0.18, -0.02]). See Appendix 4 Figures 15-20 for forest plots of absolute risk differences of antibiotic resistance for each of the six classes of antibiotics. There was significant heterogeneity across studies, with an I^2 between 96.4% and 98.8% and Cochran Q test p-value <0.05 for all studies for which meta-analysis was undertaken. No meta-analysis of multi-drug resistance to antibiotics was conducted due to insufficient numbers of studies reporting this outcome. Meta-analysis was also not undertaken for the following antibiotic classes due to insufficient numbers of studies: amphenicols (5 studies), carbapenems (1 study), cyclic esters (1 study), cyclic polypeptides (2 studies), glycylcyclines (1 study), lincosamides (3 studies), lipopeptides (1 study), nitrofurantoins (3 studies), oxazolidinones (2 studies), quinolones (3 studies), and rifamycins (1 study).

4. *Campylobacter spp. in faecal samples*

Depending on the antibiotic class, the absolute risk differences in antibiotic resistance were pooled for 7 to 11 studies (Table 9).

Table 9: Pooled absolute risk differences of antibiotic resistance for *Campylobacter* spp. isolates in faecal samples

Antibiotic class	No. studies	Pooled absolute risk difference (95% CI)
Aminoglycosides	8	-0.02 (-0.03, 0.00)
Amphenicols	7	0.00 (-0.02, 0.02)
Macrolides	11	-0.15 (-0.26, -0.04)
Penicillins	8	-0.03 (-0.08, 0.02)
Quinolones	11	-0.06 (-0.16, 0.05)
Tetracyclines	10	-0.12 (-0.20, -0.03)

Similar to the previous meta-analyses, nearly all pooled estimates across antibiotic classes were less than zero, indicating that the pooled risk of antibiotic resistance in the intervention group was lower than that in the control group. However, this was statistically significant only for macrolides (pooled absolute risk difference of -0.15 [95% CI -0.26, -0.04]) and tetracyclines (pooled absolute risk difference of -0.12 [95% CI -0.20, -0.03]). See Appendix 4 Figures 21-26 for forest plots of absolute risk differences of antibiotic resistance for each of the six classes of antibiotics. There was significant heterogeneity across studies, with an I^2 between 77.5% and 99.4% and Cochran Q test p-value <0.05 for all studies for which meta-analysis was undertaken. Meta-analysis was not undertaken for multi-drug antibiotic resistance or for resistance to the following antibiotic classes due to insufficient numbers of studies: carbapenems (1 study), cephalosporins (1 study), cyclic esters (1 study), lincosamides (3 studies), nitrofurantoins (1 study), oxazolidinones (1 study), and sulfonamides (3 studies).

5. *Campylobacter spp. in meat samples*

Meta-analyses were conducted only for three antibiotic classes, due to insufficient numbers of studies reporting resistance to the other antibiotic classes (aminoglycosides [5 studies], amphenicols [5 studies], penicillins [3 studies], and sulfonamides [1 study]). Seven studies reporting on antibiotic resistance to macrolides were pooled, nine were pooled for quinolones, and seven were pooled for tetracyclines. The pooled absolute risk difference for both macrolides and quinolones were less than 0, although neither was statistically significant (-0.04 [95% CI -0.17, 0.09], Appendix 4 Figure 27 for macrolides; -0.08 (95% CI -0.17, 0.01], Appendix 4 Figure 28 for quinolones). The pooled absolute risk difference for tetracyclines was approximately 0 (0.01 [95% CI -0.19, 0.21; Appendix 4 Figure 29) indicating that there was no difference in the pooled risk of antibiotic resistance in the intervention group compared to the control group for this antibiotic class. There was significant heterogeneity, with an I^2 between 94.1% and 97.2%. Cochran Q test p-values were all <0.05. No meta-analysis of multi-drug resistance to antibiotics was conducted due to insufficient numbers of studies reporting this outcome.

6. *Staphylococcus spp. in milk samples*

Depending on the antibiotic class, the absolute risk differences in antibiotic resistance were pooled for 6 to 10 studies (Table 10).

Table 10: Pooled absolute risk differences in antibiotic resistance for *Staphylococcus* spp. isolates in milk samples

Antibiotic class	No. studies	Pooled absolute risk difference (95% CI)
Aminoglycosides	6	-0.04 (-0.13, 0.05)
Lincosamides	7	-0.09 (-0.16, -0.02)
Macrolides	8	-0.06 (-0.10, -0.01)
Penicillins	10	-0.07 (-0.11, -0.02)
Sulfonamides	6	-0.04 (-0.07, -0.00)
Tetracyclines	9	-0.06 (-0.10, -0.01)

Similar to the meta-analyses for Enterobacteriaceae and *Enterococcus* spp., all pooled estimates across antibiotic classes were less than zero, indicating that the pooled risk of antibiotic resistance in the intervention group was lower than in the control group. This was statistically significant for all antibiotic groups except aminoglycosides. The pooled absolute risk difference of antibiotic resistance was highest for lincosamides at -0.09 (95% CI -0.16, -0.02). It was the lowest, and also not statistically significant, for aminoglycosides at -

0.04 (95% CI -0.13, 0.05). See Appendix 4 Figures 30-35 for forest plots of absolute risk differences of antibiotic resistance for each of the six classes of antibiotics. For most antibiotic classes, there was significant heterogeneity across studies, with an I^2 between 78.6% and 91.3%. However, there was very little visual or statistical heterogeneity for the antibiotic class of sulfonamides only, with an I^2 of 0.0% and p-value for Cochran Q Test of 0.68. Meta-analysis was not undertaken for multi-drug antibiotic resistance or for resistance to the following antibiotic classes due to insufficient numbers of studies: amphenicols (4 studies), cephalosporins (4 studies), cyclic polypeptides (1 study), glycopeptides (3 studies), nitrofurantoins (1 study), oxazolidinones (1 study), pseudomonic acids (1 study), quinolones (5 studies), rifamycins (2 studies), steroid antibacterials (1 study), and streptogramins (2 studies).

iv. Stratified analysis by intervention type

Due to the heterogeneity of interventions and results across studies, stratified analysis was performed by intervention type. This was done for Enterobacteriaceae in faecal samples (Appendix 4 Figures 36-42), *Enterococcus* spp. in faecal samples (Appendix 4 Figures 43-49), and *Campylobacter* spp. in faecal samples (Appendix 4 Figures 50-55). Overall, there were no clear patterns that emerged from these stratified meta-analyses. That is, absolute risk differences of antibiotic resistance were similar across all intervention types, with no one intervention being consistently associated with a higher or lower absolute risk difference when compared to the others. Of note, there were no external bans or regulation type interventions for studies reporting outcomes in Enterobacteriaceae in faecal samples. There were no voluntary restriction interventions for studies reporting outcomes in *Enterococcus* spp. or *Campylobacter* spp. in faecal samples.

Despite stratification by intervention type, visual and statistical heterogeneity remained high, indicating that intervention type alone did not explain the heterogeneity of results. Stratified analysis was not performed for Enterobacteriaceae or *Campylobacter* spp. in meat samples as these studies consisted primarily of a single intervention type (organic intervention). Similarly, all studies included in meta-analysis for *Staphylococcus* spp. in milk samples represented organic interventions, so stratified analysis could not be performed in this group either.

v. Stratified analysis by quality criteria

Of our component-based assessment of study quality (see “Study quality” below), the three criteria deemed most important were the following:

- Were animals in the intervention and comparison groups recruited from the same source population?
- Were animals included in the intervention and comparison groups recruited over the same period of time?
- Was there adequate adjustment of important potential confounders?

Due to the low numbers of studies meeting any of these three quality criteria, stratified meta-analysis within the six meta-analytic groups based on bacteria and sample type was not possible. We therefore performed a global meta-analysis for all animal studies that contained the necessary data for meta-analysis, using a pooled single effect estimate for each study (pooling within studies across all antibiotic classes, sample types, and bacterial groups). Stratification and meta-regression based on each of these three quality criteria, in addition to whether studies were published as full-text articles in peer-reviewed journals, was conducted to determine whether there was a difference in the pooled effect estimates between higher quality studies (i.e. studies meeting each of these quality criteria) and lower quality studies (Table 11).

Table 11: Stratified analysis and meta-regression of pooled absolute risk differences in overall antibiotic resistance

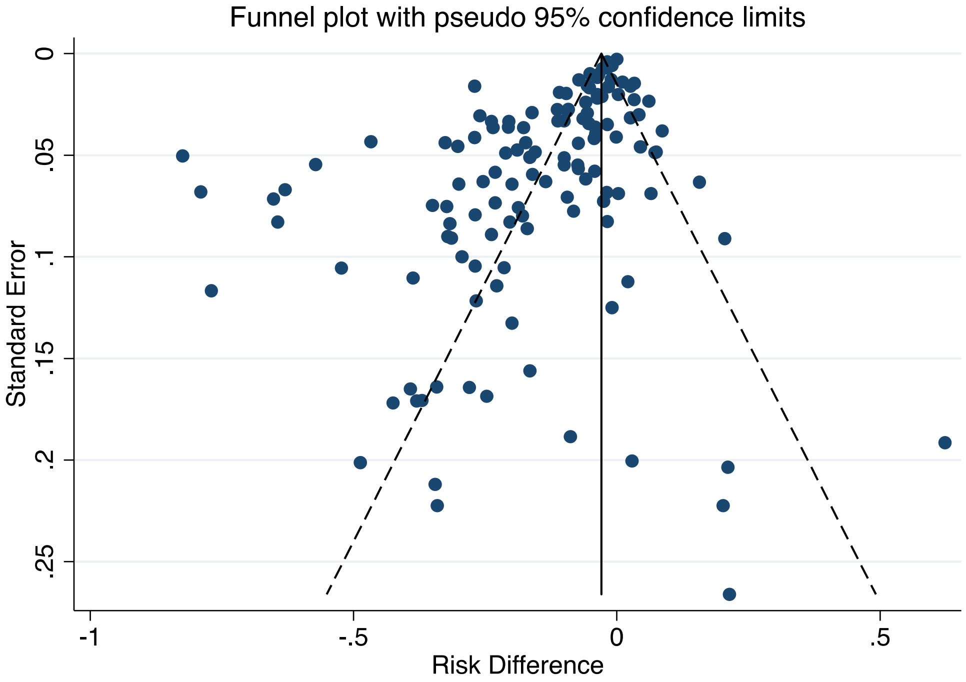
Quality criteria	Pooled absolute risk difference (95% CI) in studies meeting quality criteria [Number of studies]	Pooled absolute risk difference (95% CI) in studies not meeting quality criteria [Number of studies]	Meta-regression P-value
Groups are from same source population	-0.12 (-0.16, -0.09) [46]	-0.16 (-0.19, -0.14) [55]	0.15
Groups recruited over same period of time	-0.12 (-0.14, -0.10) [75]	-0.20 (-0.28, -0.13) [26]	0.21
Adequate adjustment of confounders	-0.10 (-0.15, -0.06) [14]	-0.16 (-0.18, -0.13) [87]	0.35
Published as a full-text article in a peer-reviewed journal	-0.15 (-0.17, -0.13) [90]	-0.10 (-0.19, -0.02) [11]	0.33

Overall, meta-analyses of both higher quality and lower quality studies demonstrated statistically significant risk reductions in antibiotic resistance in intervention compared to control groups, although effect estimates appeared to be lower for higher quality compared to lower quality studies for the three quality criteria. On meta-regression, however, none of these differences were statistically significant. There did not appear to be any differences in the effect estimates when analysis was stratified for studies that were published as full-text articles in peer-reviewed journals compared to non peer-reviewed articles such as meeting abstracts and reports found in the grey literature.

vi. Publication bias

A funnel plot was produced, including all animal studies that were meta-analyzed. Visual inspection of the funnel plot did reveal visual asymmetry (Figure 5), although Begg's test for funnel plot asymmetry was not statistically significant (p=0.16).

Figure 5: Funnel plot for animal studies included in meta-analysis



The visual asymmetry of the funnel plot suggested the potential for publication bias, despite the non-significant results on statistical testing, and therefore, sensitivity analysis using the trim and fill method was performed. There was no change in the risk reduction of antibiotic resistance in animals with interventions that reduced antibiotic use with and without imputation (both -0.13 [95% CI -0.15, -0.11]), suggesting that publication bias, if present, likely had minimal effect on our findings. Of note, the pooled risk difference seen on the funnel plot is -0.03 rather than at -0.13 as the creation of the funnel plot required the use of a fixed effects model (which gives a pooled effect estimate of -0.03). We prefer the use of a random effects model (which gives a pooled effect estimate of -0.13), recognizing the heterogeneity across studies in terms of interventions, regions, bacterial species, and antibiotics tested.

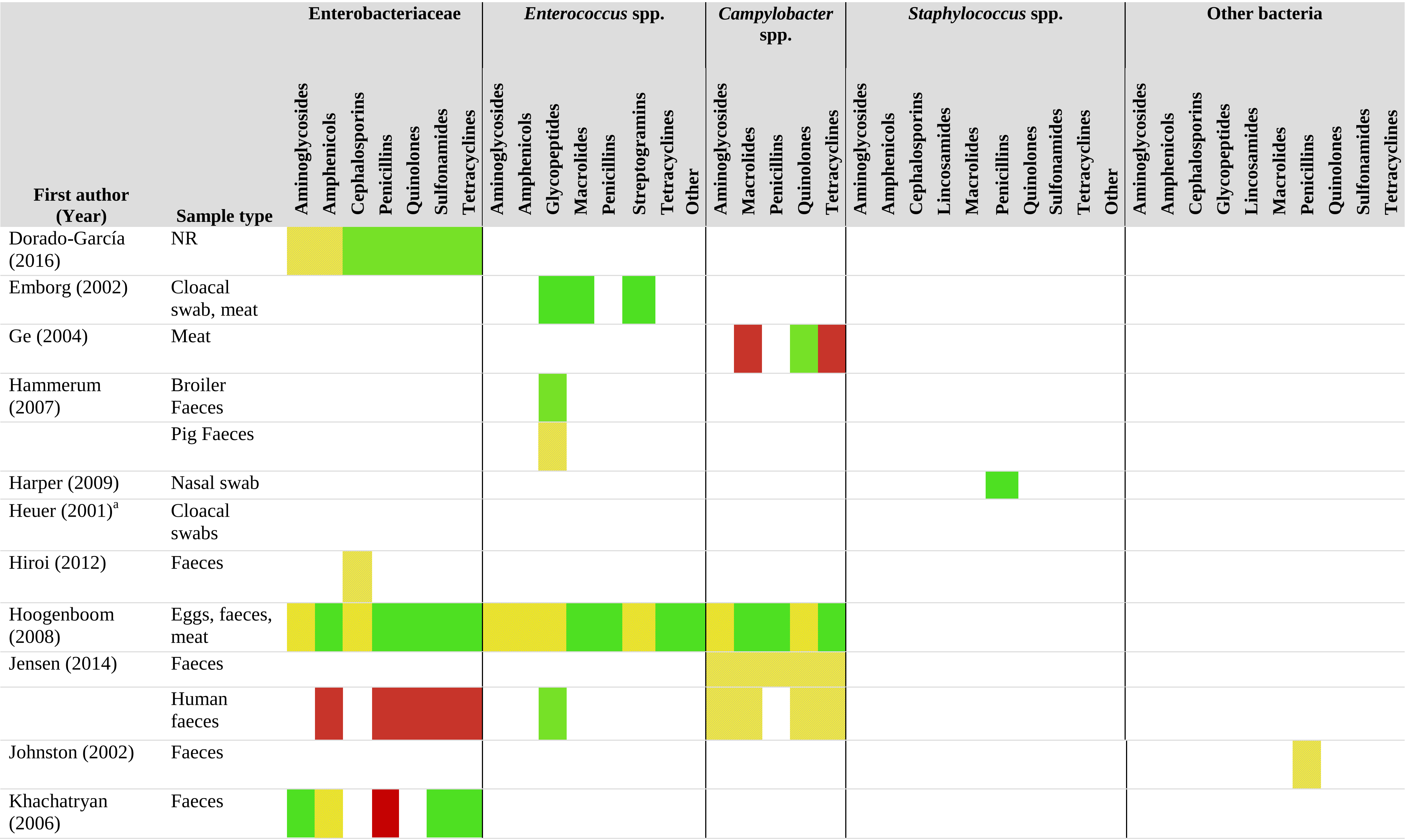
There are other factors that can contribute to funnel plot asymmetry other than publication bias. These include heterogeneity, such as when the magnitude of effect differs based on sample sizes and when there are differences in baseline antibiotic risk across studies. Given the known clinical heterogeneity of studies, with variable sample sizes, animal and bacterial groups studied, samples studied, and countries studied (all leading to probable differences in baseline risk), the funnel plot asymmetry is therefore not surprising and may not be attributable to publication bias alone.

vii. Qualitative description of study results

We conducted a qualitative descriptive analysis of the 63 studies that examined phenotypic antibiotic resistance in animals, which could not be included into the series of meta-analyses. Table 12 summarizes the study results, where red represents higher, green indicates lower, and yellow indicates no difference in the prevalence of antibiotic resistance in the intervention group compared to the comparator group respectively.

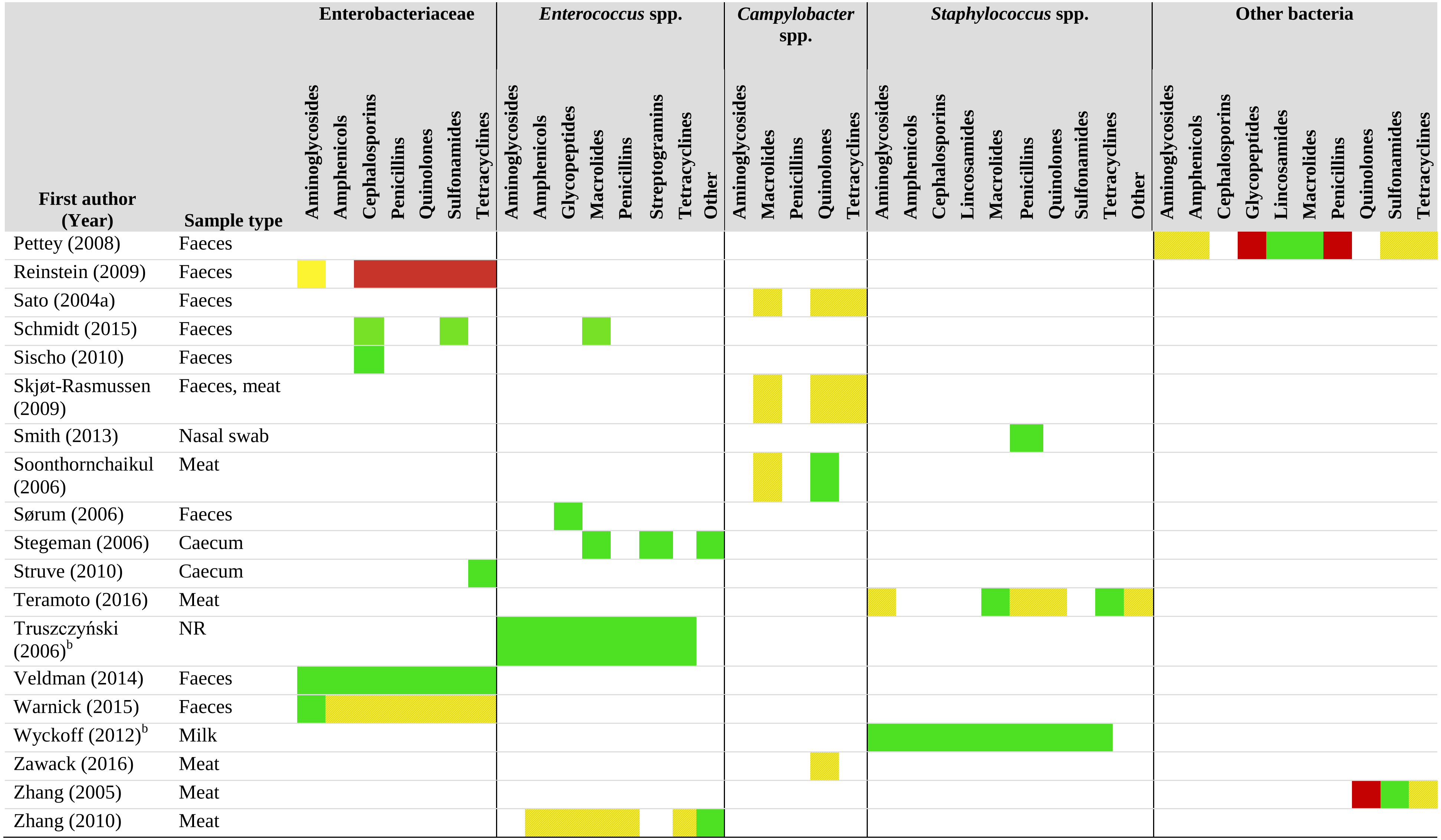
Table 12: Trends in the prevalence of antibiotic resistance by bacterial class for studies not included in meta-analyses

First author (Year)	Sample type	Enterobacteriaceae							*Enterococcus* spp.								*Campylobacter* spp.					*Staphylococcus* spp.										Other bacteria									
		Aminoglycosides	Amphenicols	Cephalosporins	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Aminoglycosides	Amphenicols	Glycopeptides	Macrolides	Penicillins	Streptogramins	Tetracyclines	Other	Aminoglycosides	Macrolides	Penicillins	Quinolones	Tetracyclines	Aminoglycosides	Amphenicols	Cephalosporins	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Other	Aminoglycosides	Amphenicols	Cephalosporins	Glycopeptides	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines
Aarestrup (2000a)	Faeces															🟩																									
Abdalrahman (2015)	Meat																										🟨														
Bager (1999)	Broiler cloacal swab,										🟩																														
	pig caecum										🟨																														
Bengtsson (2006)	NR	🟩	🟩		🟩	🟩	🟩	🟩			🟩	🟩		🟩	🟩	🟩																									
Borgen (2000)	Faeces										🟩																														
Buntenkoetter (2014)	Environment, nasal swab																					🟥	🟨	🟨	🟩	🟩	🟨	🟥	🟨	🟨	🟨										
Butaye (1999)	Chicken faeces,										🟩																														
	pig faeces										🟥																														
CIPARS (2016)	NR			🟩																																					
Coalition for Animal Health	NR				🟨	🟩		🟥			🟥			🟥	🟥			🟨		🟨	🟨																				
Docic (2003)	Rectal swab	🟩		🟩	🟩	🟩	🟩	🟩																																	
Dorado-García (2013)	Nasal swab																										🟩														
Dorado-García (2015a)	Nasal swab																										🟩														
Dorado-García (2015b)	Nasal swab																										🟩														



First author (Year)	Sample type	Enterobacteriaceae							*Enterococcus* spp.								*Campylobacter* spp.					*Staphylococcus* spp.										Other bacteria									
		Aminoglycosides	Amphenicols	Cephalosporins	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Aminoglycosides	Amphenicols	Glycopeptides	Macrolides	Penicillins	Streptogramins	Tetracyclines	Other	Aminoglycosides	Macrolides	Penicillins	Quinolones	Tetracyclines	Aminoglycosides	Amphenicols	Cephalosporins	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Other	Aminoglycosides	Amphenicols	Cephalosporins	Glycopeptides	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines
Dorado-García (2016)	NR	🟨	🟨	🟢	🟢	🟢	🟢	🟢																																	
Emborg (2002)	Cloacal swab, meat										🟩	🟩		🟩																											
Ge (2004)	Meat																	🟥		🟢	🟥																				
Hammerum (2007)	Broiler Faeces										🟢																														
	Pig Faeces										🟨																														
Harper (2009)	Nasal swab																										🟩														
Heuer (2001)[a]	Cloacal swabs																																								
Hiroi (2012)	Faeces			🟨																																					
Hoogenboom (2008)	Eggs, faeces, meat	🟨	🟩	🟨	🟩	🟩	🟩	🟩	🟨	🟨	🟨	🟩	🟩	🟨	🟩	🟩	🟨	🟩	🟩	🟨	🟩																				
Jensen (2014)	Faeces																🟨	🟨	🟨	🟨	🟨																				
	Human faeces		🟥		🟥	🟥	🟥	🟥			🟢						🟨	🟨		🟨	🟨																				
Johnston (2002)	Faeces																																					🟨			
Khachatryan (2006)	Faeces	🟩	🟨		🔴		🟩	🟩																																	

First author (Year)	Sample type	Enterobacteriaceae							*Enterococcus* spp.								*Campylobacter* spp.					*Staphylococcus* spp.										Other bacteria									
		Aminoglycosides	Amphenicols	Cephalosporins	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Aminoglycosides	Amphenicols	Glycopeptides	Macrolides	Penicillins	Streptogramins	Tetracyclines	Other	Aminoglycosides	Macrolides	Penicillins	Quinolones	Tetracyclines	Aminoglycosides	Amphenicols	Cephalosporins	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Other	Aminoglycosides	Amphenicols	Cephalosporins	Glycopeptides	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines
Kilonzo-Nthenge (2015)	Meat								🟩	🟥		🟩	🟩		🟩	🟨																									
Kola (2012)	Meat			🟨	🟨																																				
Lam (2012)	Cow faeces	🟨		🟨			🟨	🟨																			🟩														
Lebek (1979)[b]	Faeces	🟨	🟨	🟨	🟨	🟨	🟨	🟨																																	
Mathew (2001)	Faeces	🟩		🟩	🟩		🟩	🟩																																	
Mazengia (2014)	Meat	🟩	🟩	🟩	🟩	🟩	🟩	🟩																																	
Meemken (2009)	Environment																										🟩														
Millar (2007)	Meat																🟩																								
Millman (2013)	Meat	🟨		🟨	🟨	🟨	🟨	🟨																																	
Miranda (2007)	Meat									🟩	🟩	🟩	🟩		🟩	🟩																									
Miranda CD (2007)	Environment, fingerling																															🟨	🟩	🟩			🟨	🟨	🟩	🟨	🟥
Mitchell (2004)	Faeces	🟩	🟨	🟨	🟨	🟨	🟨	🟨																																	
Mollenkopf (2014)	Meat	🟨	🟨	🟨	🟨	🟨	🟨	🟨									🟨	🟨	🟨	🟨	🟨																				
Noormohamed (2014) [b]	Meat																🟨	🟨	🟨	🟨	🟨																				
Norby (2003)	Faeces																🟨	🟩		🟨	🟨																				
Nugent (2001)	Milk																					🟨	🟨	🟨	🟩	🟨	🟩	🟨	🟩	🟩											
O'Brien (2012)	Meat																										🟨														
Osadebe (2012)	Nasal swab																										🟨														
Pantosti (1999)	Meat										🟩																														
Park (2012)	Milk																					🟨		🟩	🟨	🟨	🟩			🟨	🟨										



First author (Year)	Sample type	Enterobacteriaceae							*Enterococcus* spp.								*Campylobacter* spp.					*Staphylococcus* spp.										Other bacteria									
		Aminoglycosides	Amphenicols	Cephalosporins	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Aminoglycosides	Amphenicols	Glycopeptides	Macrolides	Penicillins	Streptogramins	Tetracyclines	Other	Aminoglycosides	Macrolides	Penicillins	Quinolones	Tetracyclines	Aminoglycosides	Amphenicols	Cephalosporins	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines	Other	Aminoglycosides	Amphenicols	Cephalosporins	Glycopeptides	Lincosamides	Macrolides	Penicillins	Quinolones	Sulfonamides	Tetracyclines
Pettey (2008)	Faeces																															▨	▨		🟥	🟩	🟩	🟥		▨	▨
Reinstein (2009)	Faeces	🟨		🟥	🟥	🟥	🟥	🟥																																	
Sato (2004a)	Faeces																	▨		▨	▨																				
Schmidt (2015)	Faeces			🟩			🟩					🟩																													
Sischo (2010)	Faeces			🟩																																					
Skjøt-Rasmussen (2009)	Faeces, meat																	▨		▨	▨																				
Smith (2013)	Nasal swab																										🟩														
Soonthornchaikul (2006)	Meat																	▨		🟩																					
Sørum (2006)	Faeces										🟩																														
Stegeman (2006)	Caecum											🟩		🟩		🟩																									
Struve (2010)	Caecum							🟩																																	
Teramoto (2016)	Meat																					▨				🟩	▨	▨		🟩	▨										
Truszczyński (2006)[b]	NR								🟩	🟩	🟩	🟩	🟩	🟩	🟩																										
Veldman (2014)	Faeces	🟩	🟩	🟩	🟩	🟩	🟩	🟩																																	
Warnick (2015)	Faeces	🟩	▨	▨	▨	▨	▨	▨																																	
Wyckoff (2012)[b]	Milk																					🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩											
Zawack (2016)	Meat																			▨																					
Zhang (2005)	Meat																																						🟥	🟩	▨
Zhang (2010)	Meat									▨	▨	▨	▨		▨	🟩																									

🟥Red: higher prevalence of antibiotic resistance in intervention group compared to comparator group (i.e. increased resistance with interventions that reduce antibiotic use);

🟩 Green: higher prevalence of antibiotic resistance in comparator group compared to intervention group (i.e. decreased resistance with interventions that reduce antibiotic use);
🟨 Yellow: no difference in prevalence of antibiotic resistance in the intervention group compared to the comparator group

[a] No comparison made between intervention and control groups due to low numbers of resistant isolates

[b] Resistance not reported by individual antibiotic class

Abbreviations: NR- not reported

The results of this analysis very closely reflected the findings from the series of meta-analyses described previously. The summary of the trends found in these 63 studies was:

1. The majority of studies of antibiotic resistance in animals focused on Enterobacteriaceae.
2. For Enterobacteriaceae, *Enterococcus* spp., and *Staphylococcus* spp., the majority of studies reported a reduction in the absolute risk difference of antibiotic resistance with any intervention that aimed to reduce antibiotic use in animals, across all antibiotic classes and sample types. There were also a large number of studies that reported no statistically significant difference between intervention and control groups, and proportionately few studies that reported an increase of antibiotic resistance with interventions that aimed to reduce antibiotic use in animals.
3. Antibiotic resistance in *Campylobacter* spp. appeared to follow a different pattern compared to the other bacterial groups. Specifically, in most studies, no statistically significant difference in antibiotic resistance was detected in animals with interventions that reduced antibiotic use. There were only a small numbers of studies showing decreased antibiotic resistance with interventions that aimed to reduce antibiotic use in animals.

viii. Synthesis of results from studies reporting genetic elements

Fifty-four studies reported genotypic resistance results. See Table 13 for a description of the study characteristics, genes that were screened, and author conclusions on the genetic data for each of these studies.

Table 13: Study characteristics and conclusions from studies that report on genotypic resistance

	Intervention					Bacteria investigated						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Aarestrup (1995)			•		Broilers, egg layers		•				*vanA*	Increased risk of *vanA* in farms using avoparcin (control group)
Aarestrup (2000b)	•				Broilers, pigs		•				*erm, satA, satG, vanX*	No significant difference between risk of studied genes
Aarestrup (2002)	•				Pigs		•				*aphA, cat, erm, pbp5, tcrB, tetK, tetL, tetM, tetO, tetS, vanA, vatD, vatE*	Higher frequency of resistance genes in isolates from control group (Spain)
Abdalrahman (2015) [a]		•			Broilers, turkeys				•		*mecA*	No conclusion comparing production systems
Agersø (2013)	•				Pigs	•					$bla_{ctx\text{-}m}$, bla_{shv}, bla_{tem}, *AmpC* [b]	No significant difference between risk of studied genes
Barlow (2008)		•			Beef cows					•	*intI, intII*	Ground beef from organic cows (intervention group) had a higher frequency of integrons
Barlow (2009)		•			Beef cows	•				•	*intI, intII*	Higher frequency of integrons in conventional herds (control group)
Boerlin (2001)	•				Pigs		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Bombyk (2007)		•			Dairy cows				•		*tetK, tetL, tetM, tetO*	No significant difference in prevalence of resistance genes

Table 13 (continued)

	Intervention					**Bacteria investigated**						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Borgen (2000)	•				Chickens, humans		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Borgen (2001)	•				Broilers		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Boyer (2012)				•	Dairy cows	•					bla_{cmy-2}	Increased quantity of bla_{cmy-2} during the period when antibiotics were given
Butaye (1999)	•				Broilers, egg layers, pigs		•				*vanA*, *vanB*, *vanC1*, *vanC2*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Cicconi-Hogan (2014)		•			Dairy cows				•		*mecA*	No significant difference in frequency of the *mecA* gene
Cohen Stuart (2012)		•			Broilers	•					bla_{cmy-2}, bla_{ctx-m}, bla_{shv}, bla_{tem}	bla_{tem} was more common in meat from organic herds (intervention group)
Del Grosso (2000)	•				Pigs, poultry		•				*vanA*, *vanB*, *vanC1*	No significant difference in the prevalence of *vanA*, *vanB* and *vanC1* between studied groups
Dolejska (2011)				•	Dairy cows	•					bla_{ctx-m}	No significant difference in prevalence of resistance genes (no isolate harboring the bla_{ctx-m} gene was found)

Table 13 (continued)

	Intervention					Bacteria investigated						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Garcia-Migura (2005)		•			Broilers, pigs		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Gerzova (2015)		•			Pigs					•	*sul1*, *sul2*, *strA*, *tetA*, *tetB*, *cat*	*sul2*, *tetB* and *cat* were higher in conventional Italian farms (control group)
Guarddon (2014)		•			Beef cows, broilers, pigs	•					*tetA*, *tetB*	*tetB* was higher in meat from conventional pork and chicken farms (control group)
Halbert (2006a)		•			Dairy cows			•			*tetM*, *tetO*	No difference between *tetM* and *tetO* in the production systems
Harvey (2009)		•			Beef cows					•	*tetA*, *tetC*, *tetD*, *tetE*, *tetG*, *tetL*, *tetM*, *tetO*, *tetP*, *tetQ*, *tetS*, *tetX*	Higher prevalence of *tet* genes in conventionally raised cattle (control group)
Heuer (2002)	•	•			Broilers		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Hiki (2015)				•	Broilers	•					$bla_{cmy\text{-}2}$, $bla_{ctx\text{-}m}$, bla_{shv}, bla_{tem}, bla_{oxa}, $AmpC^{2}$	No difference was observed when comparing resistance genes
Hiroi (2012)			•		Broilers	•					$bla_{ctx\text{-}m}$, bla_{shv}, bla_{tem}, $bla_{cmy\text{-}2}$	No significant difference in prevalence of resistance genes (all ESBL-producing *E. coli* harbored $bla_{ctx\text{-}m}$ genes)

Table 13 (continued)

	Intervention					**Bacteria investigated**						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Huijbers (2015)		•			Broilers, humans	•			•		bla_{ctx-m}, bla_{shv}, bla_{tem}, bla_{cmy-2}, $AmpC^{1}$	bla_{ctx-m} was more commonly found in faeces from chickens raised in organic system (intervention group)
Kassem (2016)		•			Poultry			•			bla_{OXA-61}, *aph-3-1, tetO, cmeB*	Presence of resistance genes was comparable between the two production systems
Kieke (2006)			•		Poultry, humans		•				*vatD, vatE, ermB*	*vatE* and *ermB* were more common in meat from conventional systems (control group)
Klare (1999)	•				Chicken, turkeys, humans		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Kola (2012)		•			Broilers	•					bla_{tem}, bla_{shv}, bla_{ctx-m}	Similar ESBL gene distribution in bacteria from conventional and organic meat
Kruse (1999)	•				Broilers, turkey, pigs, humans		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Kühn (2005)	•				Beef cows, broilers, pigs, humans		•				*vanA, vanB*	No difference in the risk of vancomycin resistance determinants in human isolates

Table 13 (continued)

	Intervention					Bacteria investigated						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Lam (2012) [a]	•				Dairy cows	•	•		•		*mecA*	No comparison in the prevalence of *mecA* before and after the intervention
Lauderdale (2007)	•				Chickens		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Lenart-Boron (2016)		•			Broilers	•					*gyrA* mutations, *parC* mutations, *qepA*, *qnrA*, *qnrB*, *qnrS*	Lower prevalence of fluoroquinolone resistance determinants in organic system (intervention group)
Looft (2012)				•	Pigs	•				•	174 different genes	Feeding antibiotics increases the quantity of several antimicrobial-related genes
Lou (1995)				•	Pigs	•					*tetA*, *tetB*, *tetC*, *tetD*, *tetE*	Samples from 1994 (22 years after antibiotic withdrawal) had a lower risk of harboring *tetA*, and higher risk of harboring *tetB*
Mehboob (2003)		•			Pigs					•	*tetH*, *tetZ*, *tetC*, *tetQ*	Tetracycline resistance genes levels were lower on organic farm (intervention group)
Mollenkopf (2014)		•	•		Chickens	•		•			$bla_{cmy\text{-}2}$, $bla_{ctx\text{-}m}$, *gyrA* mutations	Increased risk of ESBL-genes in conventional farms (control group)

Table 13 (continued)

	Intervention					**Bacteria investigated**						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Obeng (2012)			•		Broiler	•					$bla_{tem,}$ bla_{shv}, bla_{cmy-2}, *floR, aphA1, aphA2, aadA, sul1, sul2, tetA, tetB, tetC, tetD, tetE, dhfrI, dhfrV, dhfrXIII, intI, intII*	Free-range systems (intervention group) had decreased risk of harboring *intI*
O'Brien (2012)			•		Pigs				•		*mecA*	No significant difference in prevalence of resistance genes (all tested isolates harbored the *mecA* gene)
O'Neill (2010)		•			Dairy cows				•		*mecA*	No difference in the prevalence of *mecA* between farms
Pantosti (1999)	•				Poultry		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Pettey (2008)			•		Pigs					•	*tet30, tetB, tetC, tetD, tetE, tetG, tetH, tetJ, tetM, tetO, tetQ, tetS, tetW, tetY, tetZ*	The absence of antibiotic use did not result in a reduction of tetracycline resistance genes
Price (2005)			•		Chickens			•			*gyrA* mutations	Antibiotic-free brand (intervention group) had lower carriage rates of mutations in *gyrA* gene
Rinsky (2013)			•		Humans				•		*mecA*	Increased risk of *mecA* in humans from conventional herds (control group)

Table 13 (continued)

	Intervention					**Bacteria investigated**						
First author (Year)	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Other**	**Genes screened**	**Conclusions on genetic data**
Sørum (2004)	•				Chicken, turkeys		•				*vanA*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Sørum (2006)	•				Broilers, turkeys, humans		•				*vanY-vanZ*	No significant difference in prevalence of resistance genes (all isolates harbored the *vanA* gene)
Tamang (2015)		•			Pigs	•					*bla*$_{ctx-m}$, *bla*$_{shv}$, *bla*$_{tem}$, *qnrA, qnrB, qnrC, qnrD, qepA, aac(60)-Ib*	Increased risk of *bla*$_{ctx-m}$ in organic farms (intervention group; though all 8 isolates harboring *bla*$_{ctx-m}$ were from the same organic farm)
Teramoto (2016) [a]		•			Chickens				•		*ermA, ermC, tetK, mecA, tetM*	No conclusion comparing production systems
Tragesser (2006) [a]				•	Dairy cows	•					*bla*$_{cmy-2}$	No conclusion comparing *bla*$_{cmy-2}$ risk in herds based on antibiotic usage
Walk (2007) [a]		•			Dairy cows	•					*bla*$_{tem}$, *bla*$_{shv}$, *bla*$_{oxa}$, *tetA, tetB, tetC, intI*	No difference between intervention and control group (the genetic composition for the *E. coli* population on farms was the same)
Wanninger (2016)[a]				•	Pigs					•	*tetC*	No conclusion comparing farms with different antimicrobial usage policies
Zhang (2005) [a]		•			Chickens					•	*tetM*	No conclusion comparing production systems

Table 13 (continued)

[a] Studies did not report on differences in prevalence of resistance genes in intervention compared to control group. These studies could not be included in Table 14.
[b] Upregulated *AmpC* production

Of these 54 studies, 46 reported the prevalence of genetic resistance elements in intervention groups compared to control groups or provided data where this could be calculated (Table 14).

Table 14: Summary of results for the 46 studies reporting prevalence of resistance genes in intervention versus control groups

Drug class/molecule	Gene/group	Total no. studies in animals and humans (human studies in parentheses)	Isolates screened[a]	No. of associations reporting prevalence or quantity of resistance genes in intervention group compared to control group in animals (and humans, in parentheses)[b]		
				Higher	Lower	No difference
Aminoglycosides	*aadA*	1	all	0	0	1
	adeA	1	all	0	1	0
	amrB	1	all	0	1	0
	aphA	1	all	0	1	0
	strA	2	all	0	0	5
Beta-Lactams	bla_{cmy-2}	3	all	0	1	2
	bla_{ctx-m}	2	all	0	0	3
	bla_{oxa}	1	all	0	0	1
	bla_{shv}	2	all	0	2	1
	bla_{tem}	2	all	1	1	1
	mecA	2	all	0	0	3
	pbp5	1	all	0	0	1
Chloramphenicol	*bcr/cmIA*	1	all	0	1	0
	cat	2	all	0	1	4
	emrD	1	all	0	1	0
	mdfA	1	all	0	1	0
	mdtH	1	all	0	1	0
	mdtL	1	all	0	1	0
	mexF	1	all	0	1	0
Copper	*tcrB*	1	all	0	2	0
Fluoroquinolones	*gyrA* mutations	2	all	0	3	2
	parC mutations	1	all	0	1	2
	qepA	1	all	0	0	1
	qnrA	1	all	0	0	1
	qnrB	1	all	0	0	1
	qnrS	1	all	0	0	1
Fosmidomycin	*rosA*	1	all	0	1	0
Glycopeptides	*vanA*	2	all	0	1	1
	vanB	1	all	0	0	1
	vanC1	1	all	0	0	1
	vanC2	1	all	0	0	1
	vanX	1	all	0	0	1
	vanY-vanZ	1	all	0	0	1
Lincosamides	*lmrA*	1	all	0	1	0
Macrolides	*acrA*	1	all	0	1	0
	erm	2(1)	all	0	1(1)	1
	mdtF	1	all	0	1	0
	mdtN	1	all	0	1	0
	mdtO	1	all	0	1	0

Table 14 (continued)

Drug class/molecule	**Gene/group**	**Total no. studies in animals and humans (human studies in parentheses)**	**Isolates screened**[a]	**No. of associations reporting prevalence or quantity of resistance genes in intervention group compared to control group in animals (and humans, in parentheses)**[b]		
				Higher	**Lower**	**No difference**
	mdtP	1	all	0	1	0
	oprA	1	all	0	1	0
	tolC	1	all	0	1	0
Streptogramins	*vatD*	2(1)	all	0	0	2(1)
	vatE	2(1)	all	0	1(1)	1
Sulfonamides	*sul1*	2	all	0	0	5
	sul2	2	all	0	2	3
Tetracyclines	*tet30*	1	all	0	0	1
	tetA	4	all	0	1	8
	tetB	3	all	0	5	4
	tetC	3	all	0	2	2
	tetD	2	all	0	0	2
	tetE	1	all	0	1	0
	tetG	2	all	0	1	1
	tetH	2	all	0	2	1
	tetJ	1	all	0	0	1
	tetK	1	all	0	0	1
	tetL	2	all	0	1	1
	tetM	3	all	0	1	3
	tetO	3	all	0	1	2
	tetP	2	all	0	1	1
	tetQ	3	all	1	3	1
	tetS	2	all	0	0	2
	tetW	1	all	0	0	1
	tetX	2	all	0	0	2
	tetZ	1	all	0	2	0
-	Other 113 genes	1	all	0	0	113
Integrons	*intI*	4	all	2	2	2
	intII	4	all	1	1	4
Total[c]		**17 (1)**[d]	**all**	5 (0)	58 (2)	201 (1)
Aminoglycosides	*aphA*	2	resistant	0	0	3
	aadA	1	resistant	0	0	1
Beta-Lactams	*AmpC*[e]	3(1)	resistant	0	0	3(1)
	$bla_{cmy\text{-}2}$	5(1)	resistant	1	0	5(1)
	$bla_{ctx\text{-}m}$	8(1)	resistant	2	0	6(1)
	bla_{oxa}	1	resistant	0	0	1
	bla_{shv}	7(1)	resistant	0	1	6(1)
	bla_{tem}	7(1)	resistant	0	0	7(1)
	mecA	2(1)	resistant	0	0(1)	2
	pbp5	1	resistant	0	0	1
Phenicol	*floR*	1	resistant	0	0	1

Table 14 (continued)

Drug class/molecule	**Gene/group**	**Total no. studies in animals and humans (human studies in parentheses)**	**Isolates screened**[a]	**No. of associations reporting prevalence or quantity of resistance genes in intervention group compared to control group in animals (and humans, in parentheses)**[b]		
				Higher	**Lower**	**No difference**
Fluoroquinolones	*aac(60)-Ib*	1	resistant	0	0	1
	gyrA mutations	1	resistant	0	1	0
	qepA	1	resistant	0	0	1
	qnrA	1	resistant	0	0	1
	qnrB	1	resistant	0	0	1
	qnrC	1	resistant	0	0	1
	qnrD	1	resistant	0	0	1
Glycopeptides	*vanA*	13(3)	resistant	0	0	16(3)
	vanB	1(1)	resistant	0	0	2(1)
	vanC1	1	resistant	0	0	2
	vanX	1	resistant	0	0	2
	vanY-vanZ	1(1)	resistant	0	0	1(1)
Macrolides	*erm*	1	resistant	0	0	2
Streptogramins	*satA*	1	resistant	0	0	2
	satG	1	resistant	0	0	2
	vatD	1	resistant	0	0	1
	vatE	1	resistant	0	0	1
Sulfonamides	*sul1*	1	resistant	0	0	1
	sul2	1	resistant	0	0	1
Tetracyclines	*tet30*	1	resistant	0	0	1
	tetA	2	resistant	2	0	0
	tetB	3	resistant	0	1	2
	tetC	3	resistant	0	0	3
	tetD	3	resistant	0	0	3
	tetE	3	resistant	0	0	3
	tetG	1	resistant	0	0	1
	tetH	1	resistant	0	0	1
	tetJ	1	resistant	0	0	1
	tetK	2	resistant	0	0	3
	tetL	2	resistant	0	1	2
	tetM	3	resistant	0	0	4
	tetO	3	resistant	0	0	4
	tetQ	1	resistant	0	0	1
	tetS	2	resistant	0	0	3
	tetW	1	resistant	0	0	1
	tetY	1	resistant	0	0	1
	tetZ	1	resistant	0	0	1
Trimethoprim	*dhfrI*	1	resistant	0	0	1
	dhfrV	1	resistant	0	0	1
	dhfrIII	1	resistant	0	0	1

Table 14 (continued)

Drug class/molecule	**Gene/group**	**Total no. studies in animals and humans (human studies in parentheses)**	**Isolates screened**[a]	**No. of associations reporting prevalence or quantity of resistance genes in intervention group compared to control group in animals (and humans, in parentheses)**[b]		
				Higher	**Lower**	**No difference**
Total[c]		**31 (6)**[d]	**resistant**	**5 (0)**	**4 (1)**	**113 (10)**

[a] Where "all" indicates that all isolates that were cultured were also examined for resistance genes and where "resistant" indicates that only phenotypically resistant isolates were examined for resistance genes

[b] A study may screen for a resistance gene from more than one animal group population. The number of associations reported in a single row (for a single gene) may be higher than the number of studies reporting on that resistance gene.

[c] A study may screen for resistance genes in all isolates as well as in phenotypically resistant isolates specifically. The total number of studies represented in this table (n=46) is not the sum of the total number of studies for the two sub-sections

[d] A study may screen for more than one resistance gene, so the total number of studies is not the sum of the numbers in the column

[e] Upregulated *AmpC* production

These 46 studies reported changes in antibiotic resistance genes with interventions that reduce antibiotics in food animals, shedding light on the mechanisms of antibiotic resistance. The 17 animal studies that performed genetic screening for both phenotypically susceptible and resistant isolates reported a total of 264 associations; 58 of these demonstrated lower prevalence of resistance genes in intervention compared to control groups, while 201 demonstrated no statistically significant difference in prevalence of resistance genes between the groups. These results corroborate the findings from the meta-analyses and the qualitative description of phenotypic results; a considerable number of studies showed that interventions that reduce antibiotic use in food animals also reduced resistance genes, especially those genes that are associated with the restricted drug. For some genes such as *bla*$_{shv}$, *tetB*, *tetQ*, this association was supported by two or more studies. Almost no studies showed an increase in resistance genes with interventions that reduced antibiotic use in animals. For many genes, there was no statistical difference in the prevalence of resistance genes in the intervention group compared to the comparator group. The lack of a statistically significant difference does not necessarily indicate the lack of association between prevalence of resistance genes and interventions, as this may be an issue of insufficient power. Individual studies had small sample sizes overall, with some studies conducting genetic screening only on a subset of isolates. Studies tended to be underpowered to detect differences in the prevalence of resistant genes between groups. Non-statistically significant trends are not represented in the table above.

The majority of studies that screened for resistant genes only in phenotypically resistant isolates showed no difference in the prevalence of resistance genes between intervention and control groups, which was expected. These results are helpful in exploring the presence of resistance genes that drive phenotypic antibiotic resistance, but do not provide information regarding overall prevalence of resistance genes in intervention and control groups. That is, phenotypically resistant isolates would be expected to have genetic resistance elements regardless of whether an intervention that reduced antibiotic use was implemented. We have presented the data from these studies not to show differences in prevalence of resistance between intervention and control groups, but to provide a comprehensive description of genetic evidence in this research area. Studies rarely evaluated genes that were not related to the restricted antibiotic, so the complete effect of any single intervention across a wide range of resistance genes remains unclear.

ix. GRADE tables

An overall assessment of the strength of evidence for the effect of interventions that reduce antibiotic use in food animals on antibiotic resistance in this population was completed using the GRADE framework (Table 15).

Table 15: PICOD 1 GRADE assessment

PICOD Question 1: For food animal populations of any age in any setting, does a restriction compared to not having that restriction of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in **food animal** populations?									
Quality assessment						**Summary of findings**		**Quality rating**	**Comments**
Design (Number of studies)	**Limitations (Risk of bias)**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Pooled estimates ***	**95% CI**		
- Observational study designs, meeting abstracts, dissertations, government reports (n=179)	- Predominantly cross-sectional study designs - Minimal adjustment for important confounders - Potential for selection bias within specific studies - Stratified analysis by three quality criteria suggested that lower quality studies may overestimate the risk reduction of antibiotic resistance associated with interventions that reduce antibiotic use in animals. However, though the effect appears less strong, the risk reduction in higher quality studies remained statistically significant. - For genetic analyses: majority were targeted gene detection in phenotypically resistant isolates only	- Direction of effect consistent though there was substantial heterogeneity observed across included studies (as measured by the I^2 statistic for pooled results)	- Interventions varied substantially and author conclusions were at times not consistent with study findings - No discrepancy between findings when meta-analyses were stratified by type of intervention - Genetic data support phenotypic data of reduced prevalence of resistance genes specific to the restricted antibiotic	- Though a large number of studies were identified, the absolute risk differences varied somewhat by bacterial family and antibiotic class under investigation - The overall trend of reduced antibiotic resistance in intervention groups compared to control groups was consistent across all bacteria, sample types, and antibiotic classes	- Potential publication bias present as measured by funnel plot asymmetry (but trim and fill sensitivity analysis did not change results) - No discrepancy between findings published in journals, abstracts, government reports	**Quinolone resistance in Enterobacteriaceae:** <u>- Faecal samples (n=17)</u> RD=-0.01 (-0.02, -0.00) <u>- Meat samples (n=12)</u> RD=-0.09 (-0.17, -0.02) **Cephalosporin resistance in Enterobacteriaceae:** <u>- Faecal samples (n=17)</u> RD=-0.01 (-0.04, 0.01) <u>- Meat samples (n=11)</u> RD=-0.07 (-0.14, 0.01) **Macrolide resistance in *Campylobacter* spp.** <u>- Faecal samples (n=11)</u> RD=-0.15 (-0.26, -0.04) <u>-Meat samples (n=7)</u> RD=-0.04 (-0.17, 0.09) **Glycopeptide resistance in *Enterococcus* spp.** <u>- Faecal samples (n=12)</u> RD=-0.22 (-0.32, -0.12) **Multi-drug resistance in Enterobacteriaceae** <u>- Faecal samples (n=19)</u> RD=-0.24 (-0.32, -0.17) <u>- Meat samples (n=14)</u>		**⊕⊕OO LOW**	- Pooled estimates showed a consistent reduction in antibiotic resistance for all interventions under investigation. These findings need to be interpreted with the caveat that there was heterogeneity with respect to animal populations under investigation, interventions, comparators, outcomes, and study design though stratification by these characteristics did not change the conclusions

						RD=-0.32 (-0.43, -0.22)		

*Limited to analyses performed on highest priority critically important antimicrobials

Abbreviations: GRADE - Grading of Recommendations Assessment, Development, and Evaluation; CI - confidence interval; RD - risk difference

Because of the observational nature of studies, the quality rating started at “Low” before any other quality criteria were considered, based on the GRADE framework. There were limitations in risk of bias, particularly with the cross-sectional study designs and minimal adjustment for potential confounders. However, these limitations did not downgrade the quality rating due to the considerable volume of evidence and the consistency of findings across many different layers of stratification (across all bacterial groups, animal species, antibiotic classes, and sample types).

c) Human studies

i. *Study characteristics*

Twenty-one studies examined antimicrobial resistance in humans (see Table 16 for a summary of study characteristics and Table 17 for detailed characteristics for individual studies).

Table 16: Summary of study characteristics for human studies

Study characteristic			**Number of studies (N=21)**
Type of article	Journal article		19
	Meeting abstract		1
	Government or organization report		1
Population studied[a]	Healthy adults		5
	Farm workers and family members		12
	Patients or “cases”		6
		Non-hospitalized only	1
		Hospitalized only	3
		Both non-hospitalized and hospitalized	3
		Unknown	1
Intervention studied	Externally imposed bans and reductions		9
	Organic Interventions		2
	Self-reported antibiotic free or related labels		5
	Voluntary reduction or withdrawal in antibiotic use		5
Sample studied[a]	Faecal		12
	Nasal swabs		8
	Urine		1
	Blood		1
	Unknown		2
Bacteria studied[a]	*Campylobacter* spp.		2
	Enterococcus spp.		8
	Enterobacteriaceae[a]		3
		Escherichia coli	3
		Salmonella spp.	1
	Staphylococcus spp.		8

[a] Categories are not mutually exclusive and studies may be included in more than one category

Table 17: Summary of the 21 studies examining antibiotic resistance in humans

			Intervention							Bacteria studied				
First author (year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Genotypic or phenotypic resistance**
Borgen (2000)*	Norway	Cross-sectional	•				Poultry producers (Faeces)	Farm	Sample (147)		•			Both
Coalition for animal health (NR)	Denmark	Longitudinal	•				(Not specified) DANMAP	(Not specified) DANMAP	(Not specified) DANMAP	•	•	•		Phenotypic
Cuny (2012)	Germany	Cross-sectional			•		Humans working or living on pig farms (Nasal swabs)	Farm	Human (202)				•	Phenotypic
Dorado-García (2015a)	The Netherlands	Longitudinal				•	Farmers, employees and family members (Nasal swabs)	Farm	Sample (158)				•	Phenotypic
Dorado-García (2015b)	The Netherlands	Longitudinal				•	Farmers, employees and family members (Nasal swabs)	Farm	Sample (206)				•	Phenotypic
Dutil (2010)	Canada	Longitudinal				•	Hospital/private clinical laboratories (Not specified)	Hospitals, clinics	Isolate (1,424)	•				Phenotypic
Gallay (2007)	France	Longitudinal	•				Hospital/private clinical laboratories (Blood, faeces, other)	Hospitals, clinics	Isolate (5,685)			•		Phenotypic
Harper (2009)	USA	Cross-sectional		•			Humans working on pig farms (Nasal and pharyngeal swabs)	Farm	Sample (71)				•	Phenotypic

Table 17 (continued)

			Intervention							Bacteria studied				
First author (year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Genotypic or phenotypic resistance**
Huijbers (2015)*	The Netherlands	Longitudinal		•			Farmers, employees, family members, farm residence (Environment, faeces & nasal swabs)	Farm	Sample humans (27) homes (75)				•	Both
Johnson (2007)	USA	Longitudinal			•		Adult hospital patients, healthy self-identified vegetarians (Faeces)	Hospital patients, healthy adult vegetarians	Isolate (530)	•				Phenotypic
Kieke (2006)*	USA	Cross-sectional			•		Hospital patients, vegetarians (Faeces, rectal swabs)	Hospital	Isolate (170)		•			Both
Klare (1999)*	Germany	Longitudinal	•				Healthy, non-hospitalized humans (Faeces)	Healthy adults	Sample (600)		•			Both
Kruse (1999)*	Norway	Longitudinal	•				Poultry farmers (Faeces)	Farm	Isolate (26)		•			Both
Kühn (2005)*	Sweden, Spain, Denmark, UK	Longitudinal	•				Healthy and hospitalized individuals, patients with enterococcal infections (Clinical samples, faeces, raw, treated, and hospital sewage)	Hospital, clinics, healthy adults, sewage facilities	Sample (522)		•			Both
Osadebe (2012)	USA	Cross-sectional				•	Humans present on the farm on the day of animal sampling (Nasal and oropharyngeal swabs)	Farm	Sample (9)				•	Phenotypic

Table 17 (continued)

			Intervention							**Bacteria studied**				
First author (year)	**Country**	**Study design**	**External ban**	**Organic**	**Antibiotic free**	**Voluntary reduction**	**Population sampled (Sample type)**	**Sample point**	**Unit of analysis (n)**	**Enterobacteriaceae**	***Enterococcus* spp.**	***Campylobacter* spp.**	***Staphylococcus* spp.**	**Genotypic or phenotypic resistance**
Rinsky (2013)*	USA	Cross-sectional			•		Humans working at pig or poultry operations, household members (Nasal swabs)	Farm	Sample (204)				•	Both
Skjøt-Rasmussen (2009)	Denmark	Cross - sectional	•				Domestically acquired human cases, travel associated human cases (DANMAP)	Hospitals, clinics	Isolate (1,023)			•		Phenotypic
Smith (2013)	USA	Longitudinal			•		Farm workers (Nasal swabs)	Farm	Sample (145)				•	Phenotypic
Sørum (2006)*	Norway	Longitudinal	•				Poultry producers (Faeces)	Farm	Sample (115)		•			Both
van den Bogaard (2000)	The Netherlands	Longitudinal	•				(Sub)urban residents (Faeces)	Healthy adults in (sub)urban areas	Sample (288)		•			Phenotypic
van den Bogaard (2001)	The Netherlands	Cross-sectional				•	Poultry farmers (Faeces)	Farm	Isolate (123)	•				Phenotypic

*Genetic data summarized in Table 13
Abbreviations: NR - not reported; UK - United Kingdom; USA - United States

The majority of studies (n=12) were in farmers and employees of farms, along with their family members. Six studies examined antibiotic resistance from patient samples provided by laboratories, either in hospital or in outpatient facilities, while five studies were in healthy adults without disease. The most common intervention studied was externally imposed bans or restrictions of antibiotic use in animals (n=9); two studies included organic interventions, five included an antibiotic-free or a similar intervention, and five consisted of voluntary limitations. The most commonly studied bacteria were *Staphylococcus* spp. and *Enterococcus* spp. Most studies tested resistance to a single class of antibiotics, specifically to penicillins for *Staphylococcus* spp. and glycopeptides for *Enterococcus* spp.

ii. Study quality

Study quality was assessed for all 21 human studies (Tables 18 and 19).

Table 18: Assessment of study quality for individual human studies

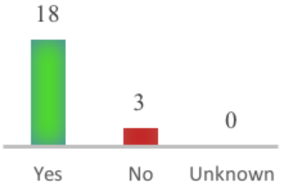
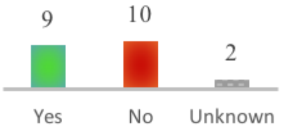
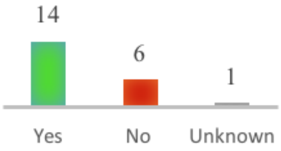
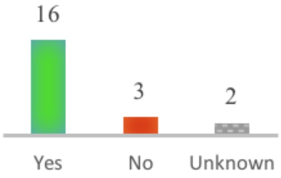
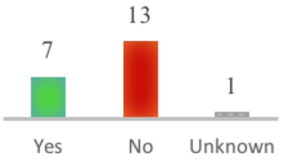
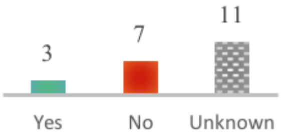
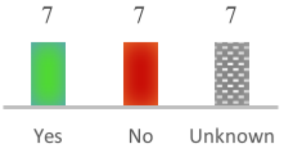
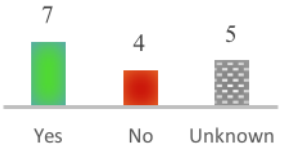
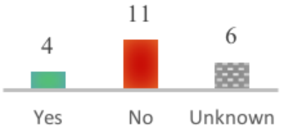
First author (Year)	Is hypothesis/aim/objective of the study clearly defined?	Are the characteristics of the animals/humans included in the study clearly described?	Are the interventions of interest clearly described?	Are the main outcomes clearly described in the Introduction or Methods section?	Does the study provide estimates of the random variability in the data for main outcomes? (I.e. SD, 95% CI, etc.)	Were the subjects included in the study representative of the entire population from which they were recruited?	Were animals/humans included in the intervention and control groups recruited from the same source population? (I.e. Were they sampled from the same population?)	Were animals/humans included in the intervention and control groups recruited over the same period of time?	Was there adequate adjustment for important confounders in the analysis?
Borgen (2000)	Yes	Unknown	Yes	Yes	Yes	Unknown	Yes	Yes	No
Coalition for Animal Health (NR)	Yes	No	Yes	Yes	No	Unknown	Unknown	N/A	No
Cuny (2012)	No	Unknown	Unknown	Unknown	No	No	Yes	Unknown	No
Dorado-García (2015a)	Yes	No	No	No	Yes	Unknown	Unknown	Yes	Unknown
Dorado-García (2015b)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Dutil (2010)	Yes	No	Yes	Yes	No	Yes	Yes	No	Unknown
Gallay (2007)	Yes	No	No	Yes	No	Yes	Yes	Yes	Unknown
Harper (2009)	Yes	Yes	No	No	Unknown	Unknown	Unknown	Unknown	Unknown
Huijbers (2015)	Yes	Yes	Yes	Yes	Yes	Yes	No	Unknown	Unknown
Johnson (2007)	Yes	Yes	No	Yes	No	Unknown	Unknown	Unknown	Yes
Kieke (2006)	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Klare (1999)	Yes	No	Yes	Yes	No	No	Yes	N/A	No
Kruse (1999)	No	No	Yes	Yes	No	Unknown	Unknown	No	No
Kühn (2005)	Yes	Yes	Yes	Yes	No	Unknown	No	No	Unknown
Osadebe (2012)	Yes	Yes	Yes	Yes	No	Unknown	Yes	Yes	Yes
Rinsky (2013)	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No

Table 18 (continued)

Skjøt-Rasmussen (2009)	Yes	No	Yes	Yes	No	Unknown	No	N/A	No
Smith (2013)	No	Yes	No	No	Yes	No	Unknown	Yes	No
Sørum (2006)	Yes	No	Yes	Yes	No	No	Yes	N/A	No
van den Bogaard (2000)	Yes	No	Yes	Unknown	No	Unknown	Unknown	N/A	No
van den Bogaard (2001)	Yes	No	No	Yes	No	Unknown	No	Unknown	No

Yes- study quality criteria met; No- study quality criteria not met; Unknown- insufficient information to assess study quality
Abbreviations: NR- year not reported; N/A- not applicable

Table 19: Numbers of human studies meeting each study quality criterion

Study quality criteria	Numbers of studies meeting study quality criteria
Is hypothesis/aim/objective of the study clearly defined?	
Are the characteristics of the animals/humans included in the study clearly described?	
Are the interventions of interest clearly described?	
Are the main outcomes clearly described in the Introduction or Methods section?	
Does the study provide estimates of the random variability in the data for main outcomes? (i.e. SD, 95% CIs, etc.)	
Were the subjects included in the study representative of the entire population from which they were recruited?	
Were animals/humans included in the intervention and control groups recruited from the same source population?	
Were animals/humans included in the intervention and control groups recruited over the same period of time?[a]	
Was there adequate adjustment for important confounders in the analysis?	

[a] Not applicable to five studies that used a historical comparator

The strengths and weaknesses of these studies were similar to the studies that examined antibiotic resistance in animals described previously. They tended to have well-described research objectives and outcome variables. The human studies did tend to have a better description of characteristics of the study sample, compared with animal studies, although only nine of 21 studies met this quality criterion. One of the most significant quality concerns was that study populations tended not to be representative of the general population with over half of studies being in farm workers. Study findings and conclusions that support a reduction of antibiotic resistance in farmers with interventions that reduce antibiotic use in animals may therefore not be generalizable to the general populations. Furthermore, the link between antibiotic use in animals and antibiotic resistance in humans is complex and the causal mechanisms are unclear and likely multi-faceted. Despite this complexity, only four studies were considered to have adequately adjusted for potential confounding variables. Other issues include the lack of information reported in the studies to demonstrate that intervention and control groups were comparable and low sample sizes, with one study including only a total of nine participants *(151)*.

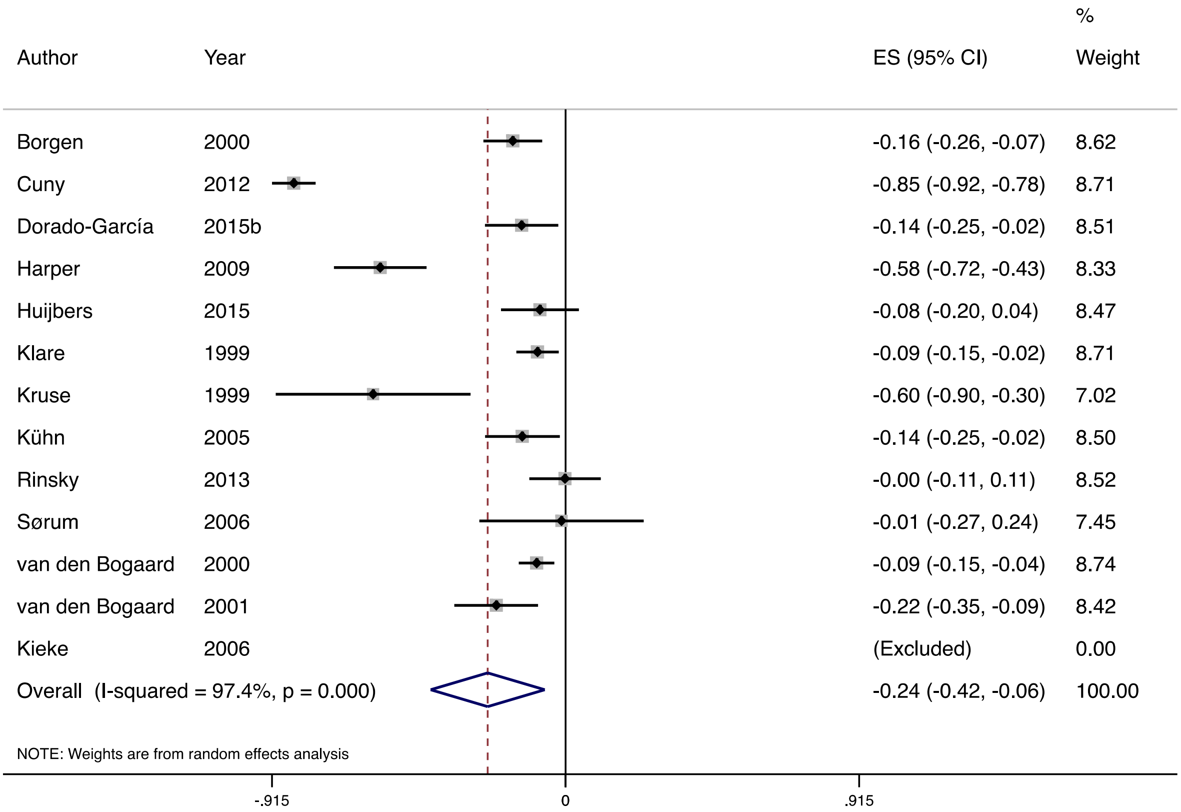
iii. Synthesis of results

There were two main groups of studies within the 21 included human studies: 1) studies examining antibiotic resistance in farmers or those with direct contact with livestock (n=12); and 2) studies examining antibiotic resistance patterns in those without direct contact with livestock animals (n=9). In the second group, the link between antibiotic use in animals and antibiotic resistance in humans was often indirect and implied. That is, authors tended to report antibiotic resistance trends in humans, using surveillance data, before and after a reduction in use or withdrawal of antibiotics in animals. Any changes to these trends were then attributed to changes in antibiotic use in animals, though many other factors may have contributed to these temporal changes and were often not considered. In both groups of studies, most, but not all, studies reported lower antibiotic resistance in the intervention versus the comparator group. Two Danish studies reported an increased prevalence of antibiotic resistance over time in the general human population, despite a restriction of antibiotic use in animals in this country *(62, 179).*

Few studies examined genetic elements of bacterial antibiotic resistance. In studies of farm workers, genetic data from two studies *(51, 184)* suggested that antibiotic resistance in bacteria isolated from these workers originated from animals. One study *(162)* suggested that methicillin-resistant *S. aureus* (MRSA) was livestock-associated on conventional farms, but that MRSA that was isolated on antibiotic-free farms did not have features of livestock association. This suggests that resistant *S. aureus* can be found on farms where there is no selective pressure from animal uses of antibiotics because of other non-animal sources of resistance. Only one study in the nine that examined antibiotic resistance trends in non-farm workers commented on the genetic origins of resistance. This study *(100)* indicated that the genetic and virulence factors of antibiotic resistant *E. coli* in humans more closely resembled *E. coli* isolates in animals compared to antibiotic susceptible isolates in humans.

Of the 21 studies that reported antibiotic resistance in humans and its association with interventions that reduced antibiotic use in animals, 13 were able to be meta-analyzed. All 13 studies showed either no difference or a lower risk of antibiotic resistance in the intervention group compared to the control group. Results from nine of the 13 studies were statistically significant, with absolute risk differences ranging from -9% to -85%. The pooled estimate of the absolute risk difference of antibiotic resistance, across all classes of antibiotics, was -0.24 (95% CI -0.42, -0.06). That is, the pooled prevalence of antibiotic resistant bacteria in humans was 24% lower in intervention groups where there was reduced used of antibiotics in animals, compared to control groups (Figure 6).

Figure 6: Pooled absolute risk differences of antibiotic resistance in humans



The study by Harper et al. *(90)* was the only abstract included in the meta-analysis, whereas the other 12 were full-text articles published in peer-reviewed journals. It did also have significant limitations in quality, but whether these limitations were due to restrictions in reporting (as it was a meeting abstract) versus actual limitations in study validity are unclear. We performed a sensitivity analysis, removing the study by Harper et al. from the meta-analysis. This resulted in only a very slight decrease to the pooled absolute risk difference (to -0.21 [95% CI -0.40, -0.03]).

Similar to the animal studies, a high degree of heterogeneity was evident, with an I^2 value of 97.4% and a p-value for the Cochran Q test <0.05. Stratified analysis by intervention group, bacterial group, or sample type was not completed due to insufficient numbers of studies.
The conclusions of the remaining eight studies that could not be meta-analyzed are summarized in Table 20.

Table 20: Study synopsis and author conclusions for the 8 human studies for which no meta-analysis could be undertaken

Author (Year)	Study synopsis	Authors' conclusions
Coalition for animal health (NR)	**Aims** This report used data available in the DANMAP reports to compare resistance patterns in humans and animals from 1997 to 2005. **Findings:** In *E. faecium* there was an increase in resistance in samples from healthy humans. Resistance to virginiamycin, vancomycin, and tetracycline increased from 29 to 54%, 0 to 2% and 8 to 16% respectively. There was a 4.3% increase in ciprofloxacin resistant *E. coli* isolates from urine. In *S. typhimurium*, resistance to ampicillin, and ciprofloxacin increased from 11 to 45% and 1 to 4% respectively between 1997 and 2005. In *C. jejuni*, resistance to ciprofloxacin and tetracycline increased from 12-14 to 28% and 9 to 25% respectively between 1997 and 2005.	The ban on the use of antibiotic growth promoters in animals in Denmark has not reduced antibiotic resistance in humans.
Dorado-García (2015b)	**Aim:** This work presents the results and experiences from an intervention study aimed at reducing MRSA in animals and humans on veal farms. **Findings:** In 193 humans assessed, the proportion of MRSA-positive people was highest in control farms (20.9%), followed by RAB-CD farms (17%). The proportion of MRSA was lowest (7.2%) in RAB farms. The difference in the proportion of MRSA between intervention groups RAB-CD and Control was only significant in the group of farmers working 20 or more hours per week ($p<0.01$).	There was no statistically significant association between prevalence of MRSA in calves and MRSA in humans (OR per 10% increase in animal prevalence=1.06, 95% CI=0.94 – 1.18, $p=0.34$). In humans working on veal farms, MRSA prevalence decreased in parallel in all study arms, with no significant difference in the decline across groups.
Dutil (2010)	**Aim:** This study examined the effect of a voluntary withdrawal of ceftiofur from hatcheries in the province of Quebec, Canada. **Findings:** Following a voluntary withdrawal of ceftiofur by hatcheries in 2005 the prevalence of ceftiofur resistance significantly decreased from 2004 to 2006 among chicken (62% to 7%; $p <0.001$) and human (36% to 8%; $p <0.0001$) *Salmonella Heidelberg* isolates. Resistance to ceftiofur among *Salmonella Heidelberg* isolates increased from 2006 to 2008 (chicken (7% to 18%) and human (8% to 12%) although this increase was not significant ($p=0.41$).	The decline in ceftiofur resistance in Quebec retail chicken meat is consistent with the voluntary withdrawal of ceftiofur use in hatcheries. There appeared to be a re-emergence of ceftiofur resistance among *E. coli* but at lower levels than baseline, when partial reinstitution of ceftiofur use in Quebec hatcheries occurred.
Johnson (2007)	**Aim:** This study used phylogenetic analysis and virulence genotyping to assess whether drug resistant human isolates resemble susceptible human isolates or resemble poultry isolates, with the purpose of identifying methods for transmission of antimicrobial resistance. **Findings:** Findings upon examination of phylogenetic group distribution and virulence gene prevalence were that drug susceptible human ExPEC isolates were different from poultry isolates. Findings also indicated that in general, drug resistant human ExPEC isolates were more similar overall to poultry isolates than to drug susceptible human isolates.	Antibiotic resistant *E. coli* isolated from humans were similar to poultry isolates, suggesting poultry origin of resistance. The presence of poultry source *E. coli* in both vegetarians and those who do consume meat products suggests that antibiotic resistant *E. coli* of poultry origin may spread through the general human population without requiring individual direct contact to poultry or poultry products.

Author (Year)	**Study synopsis**	**Authors' conclusions**
Gallay (2007)	**Aim:** This study describes trends in antimicrobial resistance in *Campylobacter* spp. isolates from 1986 to 2004. The European Union recommended that the use of fluoroquinolones in poultry be limited in 1999. **Findings:** From 2002 – 2004, resistance to quinolones decreased in *C. jejuni* isolated from both humans and broilers. The decline in resistance was less substantial in humans, suggesting that a longer period of time is required to detect the reduction in antibiotic resistance in humans after an intervention is implemented in food animals to reduce antibiotic use. No change to quinolone resistance occurred over the same time period in *C. coli* isolated from humans and boilers.	Reduction in fluoroquinolone use in broilers may result in reduced fluoroquinolone resistance in *C. jejuni* isolated from humans.
Osadebe (2012)	**Aim:** This study examined the prevalence and characteristics of *S. aureus* in pigs and pig farmers. **Findings:** Of the human participants, two humans (22%) were MRSA positive though the author did not specify whether they were from conventional or organic farms. MRSA-positive persons had other risk factors for colonization including recent hospitalization or contact with a household member with recent hospitalization. Both human strains of MRSA were similar to known healthcare associated MRSA strains. Genetic analysis suggested presence of human-animal transmission, or reverse zoonosis.	There should be improved biosafety measures to reduce spread of resistant bacteria between animals and humans.
Skjøt-Rasmussen (2009)	**Aim:** The aim of this study was to use DANMAP data to look at trends in occurrence of resistance among *C. jejuni* isolated from broiler chickens, broiler chicken meat, and human domestically acquired cases and travel-associated cases in Denmark from 1997-2007. **Findings:** The prevalence of resistance to fluoroquinolones in domestically acquired human *C. jejuni* infections was higher than that found in Danish broiler chicken meat, but similar to the prevalence found in imported chicken meat. The prevalence of antibiotic resistance was also higher in travel-associated *C. jejuni* isolated from humans compared with domestically acquired *C. jejuni* isolates.	There remains high prevalence of fluoroquinolone resistant *C. jejuni* in human infections despite the withdrawal of fluoroquinolones for animal use in Denmark. The source of these resistant organisms may be through human travel or consumption of imported chicken meat.
Smith (2013)	**Aim:** This study examined the prevalence of MRSA in pigs and farm workers on conventional and antibiotic-free pig farms in several US states. **Findings:** Out of a total of 148 farm workers, 31 were MRSA-positive (20.9%). Of these 31 individuals, 27 (87%) worked on farms where there were MRSA positive pigs present. The majority of samples came from the two farms where there was highest prevalence of MRSA in pigs. Exposure to pigs for 7 or more hours per day was associated with an increased risk for carrying MRSA-positive isolates (OR 5.2 [95% CI 4.2 – 6.5]).	Humans that have close contact with animals that are MRSA positive have a high risk of MRSA carriage themselves.

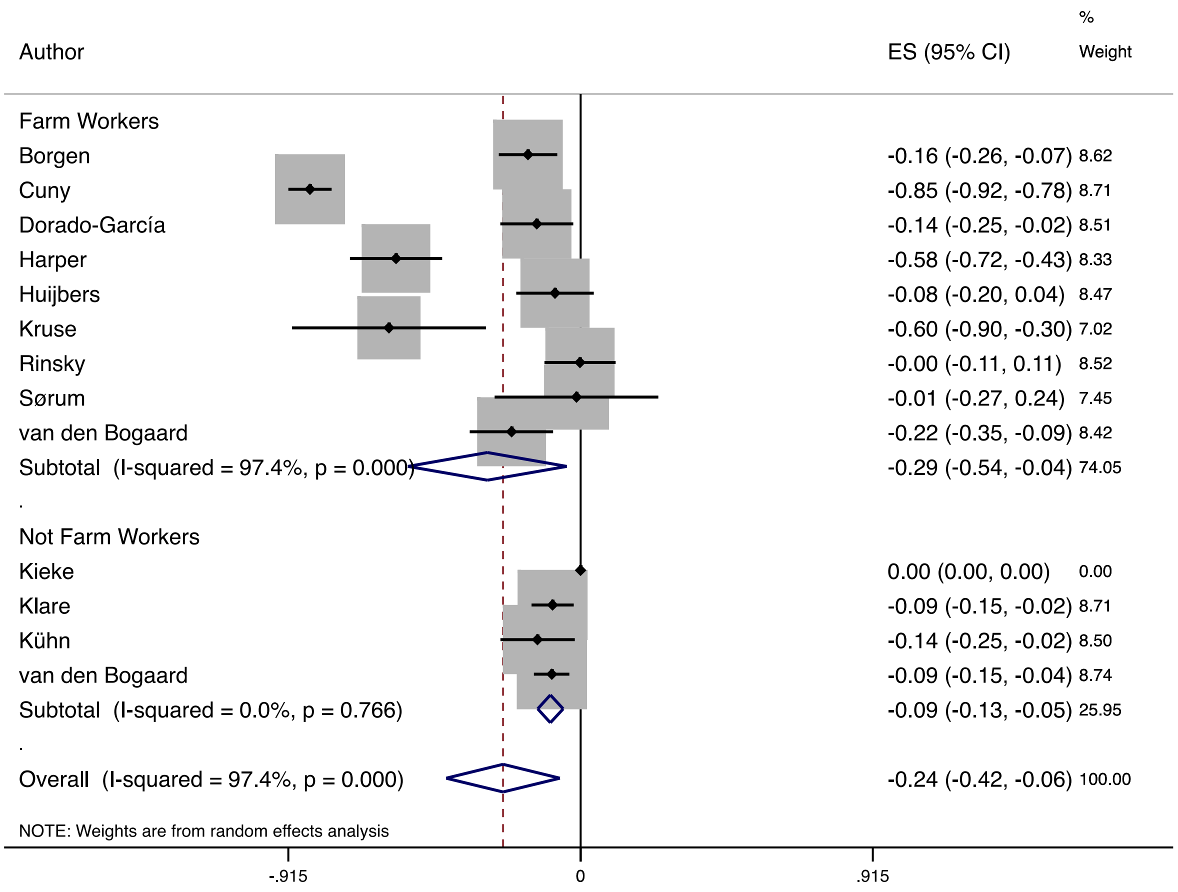
Abbreviations: AGP - Antimicrobial growth promoters; DANMAP - Danish Integrated Antimicrobial Resistance Monitoring and Research Programme; ExPEC - Extra-Intestinal Pathogenic *E. coli*; MRSA - methicillin-resistant *Staphylococcus aureus;* OR - Odds ratios; CI - Confidence intervals; RAB - Reduced antibiotic farms; RAB-CD - Reduced antibiotic-with cleaning and disinfection protocol farms.

iv. Stratified analysis by human population

We conducted stratified analysis, based on the human population. That is, meta-analysis was stratified into 1) studies examining antibiotic resistance in farm workers or those with direct contact with livestock animals;

and 2) studies examining antibiotic resistance in humans without known direct contact with livestock animals (Figure 7)

Figure 7: Stratified meta-analysis by human population

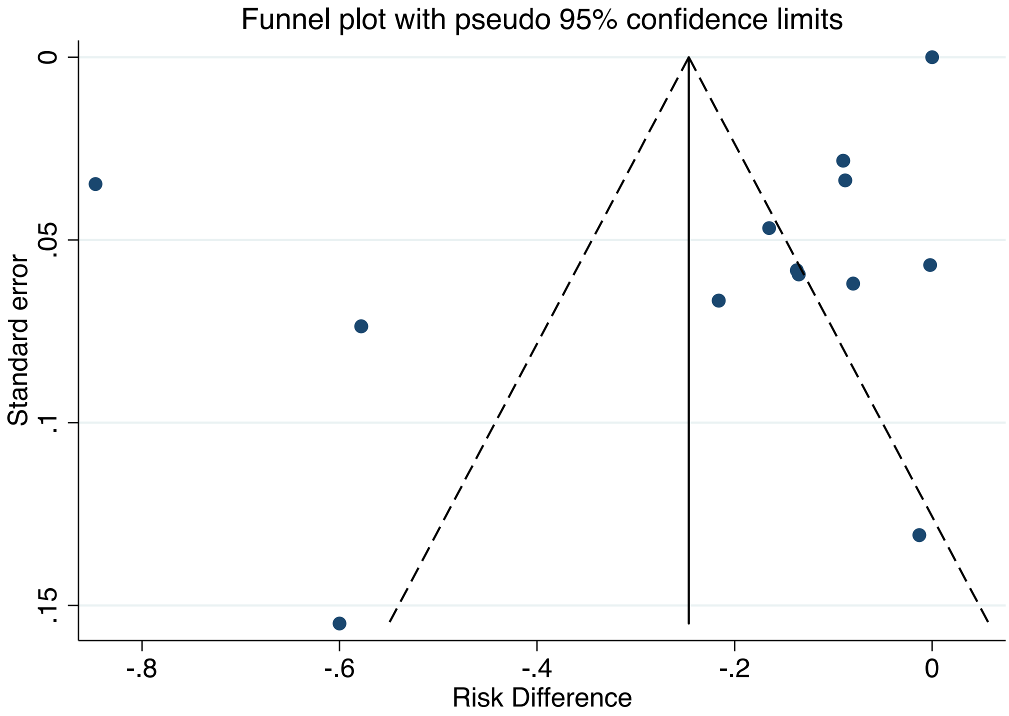


Although the pooled effect estimates were statistically significant in both populations, the pooled effect was stronger in farm workers (-0.29 [95% CI -0.54, -0.04]) compared to humans without direct contact with livestock animals (-0.09 [95% CI -0.13, -0.05]). That is, the pooled prevalence of antibiotic resistant bacteria in humans with direct contact to livestock animals was 29% lower when interventions that reduce antibiotic use in the livestock animals were implemented. For humans without direct contact to livestock animals, the pooled prevalence of antibiotic resistant bacteria was 9% lower when interventions that reduce antibiotic use in animals were implemented.

v. Publication bias

A funnel plot was produced, which included the 13 human studies that were meta-analyzed. Visual inspection of the funnel plot did reveal visual asymmetry (Figure 8), and Begg’s test for funnel plot asymmetry was statistically significant (p=0.05).

Figure 8: Funnel plot for human studies included in meta-analysis



Sensitivity analysis using the trim and fill method was performed. There was no change in the risk reduction of antibiotic resistance in humans with interventions that reduced antibiotic use in animals with (-0.27 [95% CI -0.43, -0.10]) and without (-0.24 [95% -0.42, -0.06]) imputation, suggesting that publication bias, if present, likely had minimal effect on our findings. Similar to the animal studies, known clinical heterogeneity, including heterogeneity in human populations studied, bacteria studied, and countries of origin, may have also contributed to the funnel plot asymmetry. The asymmetry therefore may not be solely attributable to publication bias.

vi. GRADE tables

An overall assessment of the strength of evidence for the effect of interventions that reduce antibiotic use in food animals on antibiotic resistance in humans was completed using the GRADE framework (Table 21).

Table 21: PICOD 2 GRADE assessment

<table>
<tr><td colspan="10"><b>PICOD Question 2:</b> Does a restriction compared to not having that restriction of use of antimicrobial agent(s) in food animals reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistant bacteria in <b>human</b> populations?</td></tr>
<tr><th colspan="6">Quality assessment</th><th colspan="2">Summary of findings</th><th rowspan="2">Quality rating</th><th rowspan="2">Comments</th></tr>
<tr><th>Design (number of studies)</th><th>Limitations (risk of bias)</th><th>Inconsistency</th><th>Indirectness</th><th>Imprecision</th><th>Publication bias</th><th>Pooled estimates</th><th>95% CI</th></tr>
<tr>
<td>- Observational study designs, meeting abstracts, government reports (n=21)

(13 studies included within a formal meta-analysis)</td>
<td>- Minimal adjustment for important confounders

- Limited reporting to demonstrate that intervention and control groups were comparable

- Potential for selection bias

- Limited generalizability (samples not representative of general population)</td>
<td>- Direction of effect consistent in pooled analysis. Heterogeneity observed across included studies (I^2=97.4%)

- Inconsistent conclusions drawn by study authors</td>
<td>- Lack of direct evidence to link limitations in antimicrobial agents in food animals and a ‘causal’ reduction in antimicrobial resistance in humans</td>
<td>- Absolute risk differences varied minimally by intervention under investigation, population studied, or antibiotic class tested for resistance</td>
<td>- Potential publication bias as measured by funnel plot asymmetry (but trim and fill sensitivity analysis did not change results)

- No discrepancy between findings when the single abstract included in meta-analysis was removed in a sensitivity analysis</td>
<td colspan="2">- <b>Pooled absolute risk differences of antibiotic resistance</b> (n=13 studies)

RD=-0.24 (-0.42, -0.06)

-<b>Stratification by the studied human population</b>

Farm workers (n=9)
RD=-0.29 (-0.54, -0.04)

Not farm workers (n=4)
RD=-0.09 (-0.13, -0.05)</td>
<td><b>⊕⊕OO
LOW</b></td>
<td>- Pooled estimates showed a reduction in antibiotic resistance in humans when interventions to reduce antibiotic use in animals were implemented. Most of the studies assessed this association within humans who had direct contact with animals. In this population, the risk reduction of antibiotic resistance was greater compared to those without direct contact with animals, though a statistically significant risk reduction was seen for both populations. These findings must be interpreted in light of statistical heterogeneity and in many cases indirect evidence.</td>
</tr>
</table>

Abbreviations: GRADE - Grading of Recommendations Assessment, Development, and Evaluation; CI - confidence interval; RD - risk difference

Because of the observational nature of studies, the quality rating started at “Low” before any other quality criteria were considered. There was direct evidence that reduction in antibiotic use in food animals was associated with a 29% reduction in antibiotic resistance in humans who have direct contact with these animals. Although the mechanisms are unclear and the evidence is more indirect, a 9% reduction in antibiotic resistance for human populations without direct contact with food animals was still demonstrated, suggesting that the benefit of interventions that reduce antibiotic use in animals may extend broadly. There were limitations in risk of bias, particularly with the observational study designs and minimal adjustment for potential confounders. These limitations did not further downgrade the quality rating due to the consistency of findings across different bacterial groups, animal species, antibiotic classes, and sample types, and because of the similarity of findings when considering animal data (PICOD 1).

Discussion

In this systematic review of 181 studies, an association was found between interventions that restrict antibiotic use and reduction in prevalence of antibiotic resistant bacteria in animals and animal products and also in the human population. When considering only the studies that examined antibiotic resistance in food animals, this association was consistent across all the studied bacterial groups, animal populations, and sample types, although the association appeared to be weaker for *Campylobacter* species. In addition, all interventions that reduced the use of antibiotics, whether the reduction was voluntary or government-imposed, and whether it included complete withdrawal of all antibiotics or limitation of use of only certain antibiotics for certain indications, seemed to be effective in reducing antibiotic resistance in animals. The magnitude of effect depended upon the antibiotic class studied, baseline antibiotic resistance, sample types, and bacterial species. Overall, reducing antibiotic use decreased prevalence of antibiotic resistant bacteria by about 15% and decreased prevalence of multi-drug resistant bacteria by between 24-32%. Furthermore, with some evidence in the literature suggesting that a ban of antibiotic growth-promoters may result in increased use of therapeutic antibiotics in animals *(211)*, it is reassuring that our meta-analysis did not reveal any evidence of increased resistance to antibiotics with such interventions.

The evidence for reduction in prevalence of antibiotic resistant bacteria in humans as a result of reduced antibiotic use in food animals was more limited and less robust than for the reduction of antibiotic resistance in the animals themselves. Meta-analysis of 13 of the 21 human resistance studies showed similar results to the meta-analyses of the animal studies. Interventions to reduce antibiotic use in animals were associated with a 24% absolute reduction in the prevalence of antibiotic resistant bacteria in humans. With nine of the 13 meta-analyzed studies being in farm workers and their direct contacts, generalizability of the findings to the general population may be limited. However, our stratified meta-analysis did suggest that reduction in antibiotic resistance in humans may extend beyond just farm workers, although the effect appears weaker for those without direct contact with animals.

Three recent systematic reviews have explored antibiotic resistance in bacteria isolated from organically—versus conventionally—farmed food animals *(212-214)*. Their conclusions were similar to the ones we have reached, although all focused only on the single type of intervention. In addition, the review by Young et al. *(214)* included studies that examined resistance in *Campylobacter* species only, while the review by Wilhelm et al. included studies that tested dairy products only *(213)*. Our systematic review is, to our knowledge, the first to include studies that examine all types of interventions that aim to reduce antibiotic use in animals, with no limitation on the type of sample collected, the animal species included, or the bacterial species tested. Therefore, this is by far the most comprehensive review on this topic. We also believe that this is the first systematic review of studies examining the association between interventions to reduce antibiotic use in food animals and changes to antibiotic resistance in bacteria in humans. Given that a key consideration for restricting antibiotic use in food animals is its potential to decrease the level of antibiotic resistance in zoonotic bacteria, this summary of evidence was commissioned by WHO to inform the development of policy recommendations for public health.

The question posed by the WHO regarding the effect on antibiotic resistance in humans, when interventions to reduce antibiotic use in food animals are undertaken, is complex. Because of the many considerations in cross-species transmission of resistant bacteria and their genetic elements, this research question cannot be easily studied and any interpretations of research findings will require a host of assumptions and inferences. There is biologic plausibility that interventions to reduce antibiotic use in food animals may reduce antibiotic resistance in humans. The results of the meta-analysis undertaken on the human studies included in our systematic review consistently suggest that antibiotic resistant bacteria can be exchanged between livestock and farm workers. It is plausible that antibiotic resistant bacteria can also be exchanged between animals and the general population; however, the evidence for this is weaker and less consistent and more indirect in the studies included in our systematic review. Transmission from food animals to the human population can occur through contaminated animal retail products *(215)*, although the risk of this may be low if animal products are adequately prepared and cooked. Resistance can also be transmitted through the environment through animal faecal matter, wastewater, and contaminated produce *(216)*.

Further adding to the complexity of the issue of antibiotic resistance is that a number of different biological drivers are involved in the selection and persistence of antibiotic resistance genes both in the natural environment and in the presence of antibiotic use *(17)*. That is, development and persistence of resistance does not depend solely on the use of antibiotics, as it can evolve naturally in bacterial populations and may provide survival benefits for bacteria in the absence of antibiotic selection pressure. With these caveats in mind, our systematic review has shown that reducing the level of antibiotic resistance in livestock populations is likely a beneficial strategy for animals and humans. Though we do not fully understand the selection and mechanisms of cross-species transmission of resistant bacteria and their genetic elements, it seems clear that the health of humans, animals, and the ecosystem are intricately linked. In this regard it is evident that a One Health approach will be required to address the problem of antimicrobial resistance *(217)*. Many jurisdictions have recognized this and have therefore implemented comprehensive surveillance of antimicrobial resistance in both the human and animal population and better co-ordination between public health and veterinary antimicrobial resistance reporting systems *(218-220)*. These tracking systems have the potential to provide high-quality information, such as detailed genomic data, necessary to track resistant bacteria in diverse settings, to better understand the links between antibiotic resistance in animals and in humans *(221)*.

There are some caveats and limitations to our systematic review and meta-analyses. Despite considerable heterogeneity across both food animal and human sets of studies, we chose to pool results through meta-analyses. This is partially because we anticipated *a priori* that there would be heterogeneity across studies, given the wide variety of settings studied and interventions described and tested. Anticipating this, we used random effect models in all of our analyses and conducted stratified analyses to explore contributors to heterogeneity. In the animal studies (PICOD 1), meta-analyses were conducted separately for groups that could not be combined in a biologically sensible way. For example, we did not pool antibiotic resistance results for all bacterial groups as their origins, resistance patterns, and isolation techniques differ widely. The bacterial species were therefore analyzed within four distinct and biologically similar groups. Even with stratification in our meta-analytic approach, there remained significant heterogeneity as demonstrated by Cochran Q test results and high I^2 values. Despite this heterogeneity, the conclusions from the meta-analyses were remarkably consistent regardless of the bacteria studied, the antibiotic class to which resistance was tested, or the sample type. Further stratification by intervention type had no effect on these associations, highlighting the robustness of these conclusions. We conducted meta-analysis on human studies (PICOD 2) without any stratification by bacterial or sample type given the low number of studies in each category. We recognized the heterogeneity of these studies, but given the consistency of findings in the series of meta-analyses that were undertaken for the animal studies, there did not appear to be significant effect modification by intervention type, antibiotic tested, or sample tested that would be a contraindication to meta-analysis.

As with any systematic review, our study is limited by the varied quality and nature of the underlying studies. While studies had clear strengths in reporting their objectives, hypotheses, and outcomes clearly, areas of deficiency included lack of description of study groups, lack of description of interventions, lack of a control group for longitudinal studies, and inadequate adjustment for potential confounders. The large majority of studies were observational, and therefore could not prove causality between reduction in antibiotic use and reduction in the prevalence of antibiotic resistant bacteria. The issue of causality was especially problematic for human studies as noted above, where linkages between bacterial resistance in food animals and bacterial resistance in humans were indirect and implied. Lastly, the majority of studies were from North America and Europe, while only one study originated from India, China, or Brazil—three of the top five global users of antibiotics in livestock and in humans *(222)*. This likely reflects the geographic areas where there has been greatest and least focus, respectively, on the reduction of antibiotic use in animals and may limit the generalizability of our findings. Furthermore, the majority of the included studies had a cross-sectional design, limiting causal inferences of associations between interventions and the reduction in antibiotic use.

The body of literature that we have identified in the systematic review has definite limitations. As noted above, these include limitations in study designs and quality and the issues of making causal inferences in this very complex area. However, we also note a substantial body of evidence that strongly suggests reduction of the prevalence of antibiotic resistant bacteria in food animals when antibiotic use is reduced in this population, and the smaller, albeit not insignificant body of evidence suggesting that such interventions may also reduce the prevalence of antibiotic resistant bacteria in humans (particularly those with direct contact with food animals). This evidence is not only substantial in its volume, but also in its consistency. The findings held regardless of bacteria studied, food animals in question, interventions implemented, samples studied, and regardless of the quality of the studies. They held when considering phenotypic resistance and genotypic resistance. The mechanisms may be indirect when considering transmission from humans to animals, but are biologically plausible. Therefore, despite the limitations posed by the quality of studies and the methodological issues and assumptions that are made in them, it would be imprudent to entirely discount this body of evidence given its coherence and consistency.

As human and veterinary medicine public health researchers, our mandate in this WHO-commissioned work has been the summarization and presentation of the evidence on the relationship between various antibiotic reduction interventions and antibiotic resistance patterns. Our findings reveal that there is a large body of evidence suggesting that interventions that restrict antibiotic use in food animals is associated with reduction in antibiotic resistance in these animals, and a smaller body of evidence showing a similar effect in humans. Decision-makers will need to determine whether these findings are sufficient to recommend widespread antibiotic reduction interventions.

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and conventional poultry, swine and beef production: a systematic review and meta-analysis. Epidemiol Infect. 2009;137(9):1217-32.
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216. Aarestrup FM. The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2015;370(1670):20140085.
217. Centers for Disease Control and Prevention. One Health 2013 [Available from: http://www.cdc.gov/onehealth/.
218. Anon. Uses of Antimicrobials in Food Animals in Canada: Impact on Resistance and Human Health. Report of the Advisory Committee on Animal Uses of Antimicrobials and Impact on Resistance and Human Health. 2002.
219. Council of the European Union. Council conclusions on the impact of antimicrobial resistance in the human health sector and in the veterinary sector – a “One Health” perspective. In: 3177th Employment SP, Health and Cosumer Affairs Council meeting, editor. Luxembourg;2012. p. 6.
220. Queenan K, Hasler B, Rushton J. A One Health approach to antimicrobial resistance surveillance: is there a business case for it? Int J Antimicrob Agents. 2016.
221. Khan LH. One Health and the Politics of Antimicrobial Resistance. Baltimore: Johns Hopkins University Press; 2016.
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APPENDIX 1: MEDLINE search strategy

#	Searches	Results
1	exp Poultry/	135574
2	exp Ruminants/	447143
3	exp Swine/	193661
4	exp Bees/	9624
5	exp Fishes/	154782
6	exp Seafood/	11624
7	exp Mollusca/	52115
8	exp Crustacea/	35148
9	(food animal* or farm* or production animal* or livestock or feedlot* or animal feeding operation* or AFO or CAFO).kw,tw.	78843
10	(ruminant* or cattle or bovine or cow* or beef or heifer* or steer* or calf or calves or sheep or ovine or caprine or goat* or equine or horse* or lepine or rabbit* or deer or elk or game or buffalo or bison or swine or pork or pig* or hog* or boar*).kw,tw.	1164249
11	(chicken* or broiler* or turkey* or duck* or geese or goose or poultry or fowl or avian).kw,tw.	175957
12	(bee or bees or honeybee* or apiary or apicultur*).kw,tw.	14422
13	((farm* or aquaculture) adj2 (fish or shellfish or seafood or amberjack or arapaima or asp or atipa or barb or barramundi or bass or beluga or bluefin or bluefish or bocachico or bonythongue or bream or bullhead or carp or catfish or char or cichlid or cobia or cod or dorada or eel* or gourami or guapote or grouper or halibut or lai or loach or mackerel or mandarin fish or meagre fish or milkfish or mojarra or mullet or mudfish or nori nei or perch or pejerrey or pike or porgy or pompano or red drum or roach or roho labeo or salmon or sampa or seabass or seabream or snakehead or snapper or snook or sole or spinefood or sturgeon or sweetfish or tench or tilapia or trout or tuna or turbot or vendace or whitefish)).kw,tw.	3753
14	((farm* or aquaculture) adj2 (shrimp or prawn* or crayfish or lobster* or crab*)).kw,tw.	615
15	((farm* or aquaculture) adj2 (abalone or bivalve* or clam* or carpet shell or cockle* or corbicula or geoduck or mussel* or oyster* or periwinkle* or quahog or sand gaper* or scallop* or shellfish or tagelus or venus)).kw,tw.	439
16	aquaculture.kw,tw.	6172
17	Aquaculture/	4788
18	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17	1832370
19	drug resistance, microbial/ or exp drug resistance, bacterial/	122825
20	((antibacterial or anti-bacterial or antibiotic or anti-biotic or antimicrobial or anti-microbial) adj2 (resistan* or susceptib* or minimum inhibitory concentration)).kw,tw.	52123
21	((aldesulfone or amdinopenicillin* or amikacin or aminocyclitol* or aminoglycoside* or aminopenicillin* or amoxicillin* or ampicillin or amphenicol* or ansamycin* or antipseudomonal or antistaphylococcal or apramycin or arbekacin or aspoxicillin or avilamycin or avoparcin or azalide or azidocillin or azithromycin or azlocillin or aztreonam or bacampicillin or bacitracin or baquiloprim or bekanamycin or benzylpenicillin or biapenem or bicozamycin or bicyclomycin* or brodimoprim or calcium aminosalicylate or capreomycin or carbadox or carbapenem* or carbenicillin or carboxypenicillin* or carindacillin or carumonam or cef* or cepha* or chloramphenicol or chlortetracycline or cinoxacin or ciprofloxacin or clarithromycin or clindamycin or clofazimine or clometocillin or clomocycline or cloxacillin or colistin or cyclic ester* or cyclic polypeptide* or cycloserine or dalbavancin or dalfopristin or danofloxacin or dapsone or daptomycin or demeclocycline or diaminopyrimidine* or dibekacin or dicloxacillin or difloxacin or dirithromycin or dihydrostreptomycin or doripenem or doxycycline or dihydrofolate reductase inhibitor* or enoxacin or enramycin or enrofloxacin or	93911

	epicillin or ertapenem or erythromycin or ethambutol or ethionamide or faropenem or fleroxacin or flomoxef or florphenicol or flucloxacillin or flumeqin* or fluoroquinolone* or flurithromycin or fosfomycin or framycetin or furaltadone or furazolidone or fusidic acid or gamithromycin or garenoxacin or gatifloxacin or gemifloxacin or gentamicin or glycopeptide* or glycylcycline* or gramicidin or grepafloxacin or hetacillin or ibafloxacin or iclaprim or imipenem or ionophore* or isepamicin or isoniazid or josamycin or kanamycin or ketolilde* or kitasamycin or lasalocid or latamoxef or levofloxacin or lincomycin or lincosamide* or linezolid or lipopeptide* or lomefloxacin or loracarbef or lymecycline or macrolide* or maduramycin or marbofloxacin or mecillinam or meropenem or metacycline or metampicillin or methicillin or meticillin or metronidazole or mezlocillin or midecamycin or miloxacin or miocamycin or minocycline or mirosamycin or monensin or monobactam* or morinamide or moxifloxacin or mupirocin or nafcillin or nalidixic acid or narasin or neomycin or netilmicin or nifurtoinol or nitrofur* or nitroimidazole* or norfloxacin or novobiocin or ofloxacin or oleandomycin or orbifloxacin or oritavancin or ormosulfathiazole or ornidazole or orthosomycin* or oxacillin or oxazolidinone* or oxolinic acid or oxytetracycline or panipenem or para-aminosalicylic acid or paromomycin or pazufloxacin or pefloxacin or penamecillin or penethatamate or penicillin* or penimepicycline or phenethicillin or pheneticillin or phenicol* or phenoxypenicillin* or phenoxymethylpenicillin or phthalylsulfathiazole or pipemidic acid or piperacillin or pirlimycin or piromidic acid or pivampicillin or pivmecillinam or pleuromutilin* or polymixin* or polymyxin* or polypeptide* or pristinamycin or propicillin or protionamide or prulifloxacin or pseudomonic acid* or pyrazinamide or pyrimethamine or quinolone* or quinoxaline* or quinupristin or retapamulin or ribostamycin or rifa* or riminofenazine* or rokitamycin or rolitetracycline or rosoxacin or roxithromycin or rufloxacin or salfadoxine or salinomycin or semduramicin or sisomicin or sitafloxacin or sodium aminosalicylate or sparfloxacin or spectinomycin or spiramycin or streptoduocin or streptogramin* or streptomycin or sulbenicillin or sulfachlorpyridazine or sulfadi* or sulfafurazole or sulfaisodimidine or sulfisoxazole or sulfon* or sulfaguanidine or sulfam* or sulfon* or sulfanilamide or sulfafurazole or sulfalene or sulfam* or sulfanilamide or sulfap* or sulfaquinoxaline or sulfath* or sultamicillin or talampicillin or teicoplanin or telavancin or telithromycin or temafloxacin or temocillin or terdecamysin or terizidone or tetracycline* or tetroxoprim or thiamphenicol or tiamulin or ticarcillin or tigecycline or tildipirosin or tilmicosin or tinidazole or tiocarlide or tobicillin or tobramycin or trimethoprim or troleandomycin or trovafloxacin or tulathromycin or tylosin or tylvalosin or valnemulin or vancomycin or virginiamycin) adj2 (resistan* or susceptib* or minimum inhibitory concentration)).kw,tw.	
22	exp Drug Resistance/	273591
23	exp Anti-Bacterial Agents/ or exp Animal Feed/	654369
24	(antibacterial or anti-bacterial or antibiotic or anti-biotic or antimicrobial or anti-microbial).kw,tw.	291134
25	23 or 24	791722
26	22 and 25	102558
27	AMR.kw,tw.	1504
28	19 or 20 or 21 or 26 or 27	188126
29	((reduc* or decreas* or restrict* or limit* or ban or bans or banning or eliminat* or control* or regulat* or less* or cut* or scale* or scaling or down* or taper*) adj5 ("use" or usage or utilization or dose* or dosage or administ* or prescri*)).kw,tw.	427628
30	(organic or (antibiotic adj2 free) or without antibiotic* or without antimicrobial*).kw,tw.	216036
31	29 or 30	640063
32	18 and 28 and 31	849
33	remove duplicates from 32	835

Notes on search terms:

- Antibiotic-related keywords were derived from the WHO List of Critically Important Antimicrobials for Human Medicine (http://apps.who.int/iris/bitstream/10665/77376/1/9789241504485_eng.pdf) and the OIE List of Antimicrobial Agents of Veterinary Importance (http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/Eng_OIE_List_antimicrobials_May2015.pdf).
- Keywords for species used in aquaculture were derived from the FAO Fisheries list of animal species used in aquaculture at http://www.fao.org/docrep/W2333E/W2333E00.htm.

APPENDIX 2: Grey literature search strategy

The following websites/agencies/documents were included in our grey literature search:

1. CANADA

1.1 Canadian Antimicrobial Resistance Alliance (http://www.can-r.com)

Manuscripts

1.2 Public Health Agency
Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) (http://www.phac-aspc.gc.ca/cipars-picra/index-eng.php)
Canadian Antimicrobial Resistance Surveillance System Reports 2013 – 2015
Canadian Nosocomial Infection Surveillance Program (CNISP) (http://www.phac-aspc.gc.ca/nois-sinp/survprog-eng.php)

- Surveillance projects
- Publications

1.3 Health Canada
Antimicrobial resistance section (http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/index-eng.php)

1.4 Canadian Institutes of Health Research (http://www.cihr-irsc.gc.ca)
Health Services and Policy Research

- Publications

Population and Public Health

- Publications

2. DENMARK

DANMAP (http://www.danmap.org/)

- Reports

3. THE NETHERLANDS

3.1 MARAN (http://www.wageningenur.nl/en/Research-Results/Projects-and-programmes/MARAN-Antibiotic-usage.htm)

- References

3.2 Sda

- Associations between antimicrobial use and the prevalence of resistant micro-organisms (http://www.autoriteitdiergeneesmiddelen.nl/Userfiles/rapport%20ab%20en%20resistentie/def-engels-rapport-abgebruik-en-resistentie-0516.pdf)

3.3 Government of the Netherlands

- Reduced and Responsible: use of antibiotics in food-producing animals in the Netherlands (https://www.government.nl/documents/leaflets/2014/02/28/reduced-and-responsible-use-of-antibiotics-in-food-producing-animals-in-the-netherlands)

4. SWEDEN

SVARM

- Reports (http://www.sva.se/en/antibiotika/svarm-reports)

5. AUSTRALIA

JETACAR (http://www.health.gov.au/internet/main/publishing.nsf/Content/health-pubs-jetacar-cnt.htm)

6. UNITED KINGDOM

Antimicrobial Resistance (https://www.gov.uk/government/collections/antimicrobial-resistance-amr-information-and-resources#strategic-publications)

  - Strategic publications

7. EUROPEAN UNION

7.1 Health and Food Safety

AMR (http://ec.europa.eu/dgs/health_food-safety/amr/action_eu/index_en.htm)
Projects, Studies and Related Information (http://ec.europa.eu/dgs/health_food-safety/amr/projects/index_en.htm)

7.2 European Centre for Disease Prevention and Control (ECDC)

European Antimicrobial Resistance Surveillance Network (EARS-Net) (http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Pages/index.aspx)
European Surveillance of Antimicrobial Consumption (ESAC-Net) (http://ecdc.europa.eu/en/activities/surveillance/ESAC-Net/Pages/index.aspx)

7.3 European Food Safety Authority (EFSA)

Antimicrobial resistance (http://www.efsa.europa.eu/en/topics/topic/amr)

  - Completed work

7.4 European Medicines Agency (EMA)

European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp)
Antimicrobial Resistance (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_001686.jsp&mid=WC0b01ac05807a4e0d)

  - ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals

8. CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC)

Antibiotic/Antimicrobial Resistance (https://www.cdc.gov/drugresistance/)

  - Digital Materials (https://www.cdc.gov/drugresistance/resources/digital_materials.html)
  - Publications (https://www.cdc.gov/drugresistance/resources/publications.html)
  - National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) (http://www.cdc.gov/narms/index.html)
    - Publications section
  - Translatlantic Task Force on Antimicrobial Resistance (TATFAR) (https://www.cdc.gov/drugresistance/tatfar/index.html)
    - Links and Resources
  - Interagency Taskforce on Antimicrobial Resistance (ITFAR) (http://www.cdc.gov/drugresistance/itfar/index.html
    - ITFAR Link and Resources

9. US FOOD AND DRUG ADMINISTRATION (FDA)

Antimicrobial Resistance (http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/)
- Guidance for Industry #209

10. JOINT PROGRAMMING INITIATIVE ON ANTIMICROBIAL RESISTANCE (http://www.jpiamr.eu/)

Library
Workshop reports
Papers

11. WORLD ANTIMICROBIAL RESISTANCE CONFERENCE US (http://www.terrapinn.com/conference/antimicrobial-resistance-congress-usa/index.stm)

12. WORLD HEALTH ORGANIZATION (WHO)

12.1 IRIS Repository (http://apps.who.int/iris/)
Search “antimicrobial resistance”

Antimicrobial use in aquaculture and antimicrobial resistance
- Antimicrobial resistance and rational use of antimicrobial agents (EM/RC49/8)
- Use of antimicrobials in food animals (weekly epidemiological record, no. 33, 18 August 2000)
- Impacts of antimicrobial growth promoter termination in Denmark
- Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment
- Second Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Management options
- The Medical Impact of Antimicrobial Use in Food Animals. Report of a WHO Meeting. Berlin, Germany, 13-17 October 1997
- Containing Antimicrobial Resistance (WHO/CDS/CSR/DRS/99/2)
- WHO Scientific working group on monitoring and management on bacterial resistance to antimicrobial agents (WHO/CDS/BVI/95.7)
- Regional strategy on prevention and containment of antimicrobial resistance 2010-2015
- Use of quinolones in food animals and potential impact on human health (WHO/EMC/ZDI/98.12)

12.2 Strategic and Technical Advisory Group (STAG) on antimicrobial resistance (http://www.who.int/antimicrobial-resistance/events/stag/en/)
6th meeting 11 May 2016
5th meeting 23-24 November 2015
4th meeting 24-25 February 2015
3rd meeting 17 October 2014
2nd meeting 14-16 April 2014
1st meeting 19-20 September 2013

12.3 International Clinical Trials Registry Platform (http://apps.who.int/trialsearch/Default.aspx)
List by “Health Topics”
- Antimicrobial Resistance
- Epidemiology

13. INTERNATIONAL VETERINARY INFORMATION SERVICE (http://www.ivis.org/home.asp)

Search “antimicrobial resistance”

14. EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECITOUS DISEASES (ESCMID)

14.1 eLibrary (https://www.escmid.org/escmid_publications/escmid_elibrary/)
Search "antimicrobial use animals"

- Checked all items until achieve 50% of relevance scale

14.2 Conference on Reviving Old Antibiotics
(https://www.escmid.org/research_projects/escmid_conferences/past_escmid_conferences/reviving_old_antibiotics/)
Final Programme

14.3 The Lancet/ESCMID Conference on healthcare-associated infections and antimicrobial resistance
(https://www.escmid.org/research_projects/escmid_conferences/past_escmid_conferences/hai_and_ab_resistance/)

15. ReACT (http://www.reactgroup.org/)

Policy and Reports

16. CONSUMERS INTERNATIONAL

WCRD 2016: Antibiotic Resistance (http://www.consumersinternational.org/our-work/wcrd/wcrd-2016/)

- WCRD 2016 Resource Pack

17. WORLD ORGANIZATION FOR ANIMAL HEALTH (OIE)

Antimicrobial Resistance (http://www.oie.int/en/for-the-media/amr/)
OIE Global Conference on the Prudent Use of Antimicrobial Agents for Animals

- Presentations / Abstracts (http://www.oie.int/eng/A_AMR2013/presentations.htm)

18. ANTIMICROBIAL RESISTANCE ONE HEALTH COLLOQUIUM

Available on
https://www.chathamhouse.org/sites/files/chathamhouse/field/field_document/AMR%20%20One%20Health%20Colloquium%20Meeting%20Summary%20jm-er%20ao%20-%2028%20April%2015.pdf

19. FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS (FAO)

Antimicrobial Resistance (http://www.fao.org/antimicrobial-resistance/en/)

- Publications section
- Uso de antimicrobianos en animales de consumo (http://www.fao.org/3/a-y5468s.pdf)
- Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials (ftp://ftp.fao.org/docrep/fao/010/i0204e/i0204e00.pdf)
- CODEX Alimentarius
    - Guidelines for risk analysis of foodborne antimicrobial resistance CAC/GL 77- 2011 (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BGL%2B77-2011%252FCXG_077e.pdf)
    - Code of practice to minimize and contain antimicrobial resistance CAC/RCP 61-2005 (http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BRCP%2B61-2005%252FCXP_061e.pdf)

20. ANIMAL HEALTH INSTITUTE

Animal Antibiotics (http://www.ahi.org/issues-advocacy/animal-antibiotics/)

- The Danish experience (http://www.ahi.org/wp-content/uploads/2011/06/Pork-Check-off-DanishExperience1.pdf)
- Political bans on antibiotics are counterproductive (http://www.ahi.org/Files/Antibiotics%20in%20Livestock/H.%20Danish%20experience.pdf)

21. AMERICAN VETERINARY MEDICAL ASSOCIATION (AVMA)

Antimicrobial Resistance FAQs (https://www.avma.org/KB/Resources/FAQs/Pages/Antimicrobial-Use-and-Antimicrobial-Resistance-FAQs.aspx)

22. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (NIAID)

NIAID's Antibacterial Resistance Program: Current Status and Future Directions (http://www.niaid.nih.gov/topics/antimicrobialResistance/Documents/ARstrategicplan2014.pdf)

23. CLINICALTRIALS.GOV

Search “Antimicrobial Animal”

24. INNOVATIVE MEDICINES INITIATIVE (IMI)

Combating antimicrobial resistance in Europe (COMBACTE) (http://www.imi.europa.eu/content/combacte)

- Projects

25. LIST OF CONFERENCE PROCEEDINGS FROM SCIENTIFIC MEETINGS:

Proceedings/presentations from the following conferences/meetings were manually reviewed and abstracts were selected based on the eligibility criteria described in the methods section of the report:

ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens
Denmark, 2008

2nd ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans and the Environment
Canada, 2010

3rd ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens in Animals, Humans and the Environment
France, 2012

4th ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens
United States, 2015

2009 - ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals
England, 2009

2011 - Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications
United States, 2011

3rd ASM-ESCMID Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications
Denmark, 2013

4th ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications
United States 2015

American Society for Microbiology 110th – 115th General Meetings

ASM-ESCMID International Workshop on Dermatological Infections and Food-borne Diseases
United States, 2015

2010 International Conference on Antimicrobial Research
Spain, 2010

2012 International Conference on Antimicrobial Research
Portugal, 2012

2014 International Conference on Antimicrobial Research
Spain, 2014

2016 International Conference on Antimicrobial Research
Spain, 2016

National Foundation for Infectious Diseases 2002 Conference on Antimicrobial Resistance
United States, 2002

National Foundation for Infectious Diseases 2003 Conference on Antimicrobial Resistance
United States, 2003

National Foundation for Infectious Diseases 2004 Conference on Antimicrobial Resistance
United States, 2004

National Foundation for Infectious Diseases 2005 Conference on Antimicrobial Resistance
United States, 2005

National Foundation for Infectious Diseases 2006 Conference on Antimicrobial Resistance
United States, 2006

National Foundation for Infectious Diseases 2007 Conference on Antimicrobial Resistance
United States, 2007

National Foundation for Infectious Diseases 2008 Conference on Antimicrobial Resistance
United States, 2008

National Foundation for Infectious Diseases 2010 Conference on Antimicrobial Resistance
United States, 2010

2015 Meeting of the Transatlantic Taskforce on Antimicrobial Resistance
Luxembourg, 2015

CDC 64th Epidemic Intelligence Service
United States, 2015

14th International Congress on Infectious Diseases
United States, 2010

15th International Congress on Infectious Diseases
Thailand, 2012

16th International Congress on Infectious Diseases
South Africa, 2014

17th International Congress on Infectious Diseases
India, 2016

2007 International Meeting on Emerging Diseases and Surveillance
Austria, 2007

2009 International Meeting on Emerging Diseases and Surveillance
Austria, 2009

2011 International Meeting on Emerging Diseases and Surveillance
Austria, 2011

2013 International Meeting on Emerging Diseases and Surveillance
Austria, 2013

2014 International Meeting on Emerging Diseases and Surveillance
Austria, 2014

22nd World Buiatrics Congress
Germany, 2002
23rd World Buiatrics Congress
Canada, 2004

24th World Buiatrics Congress
France, 2006

25th World Buiatrics Congress
Hungary, 2008

26th World Buiatrics Congress
Chile, 2010

27th World Buiatrics Congress
Portugal, 2012

28th World Buiatrics Congress
Australia, 2014

2010 National Mastitis Council Annual Meeting
United States, 2010

2011 National Mastitis Council Annual Meeting
United States, 2011

2012 National Mastitis Council Annual Meeting
United States, 2012

2013 National Mastitis Council Annual Meeting
United States, 2013

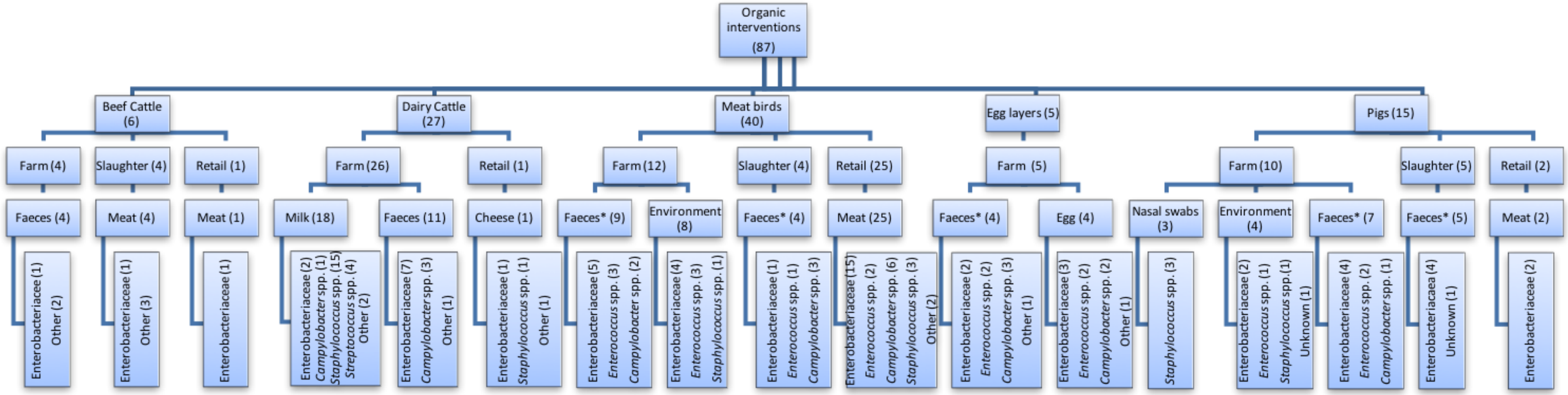
2014 National Mastitis Council Annual Meeting
United States, 2014

2015 National Mastitis Council Annual Meeting
United States, 2015

2011 International Meeting on Neglected Tropical Diseases
United States, 2011

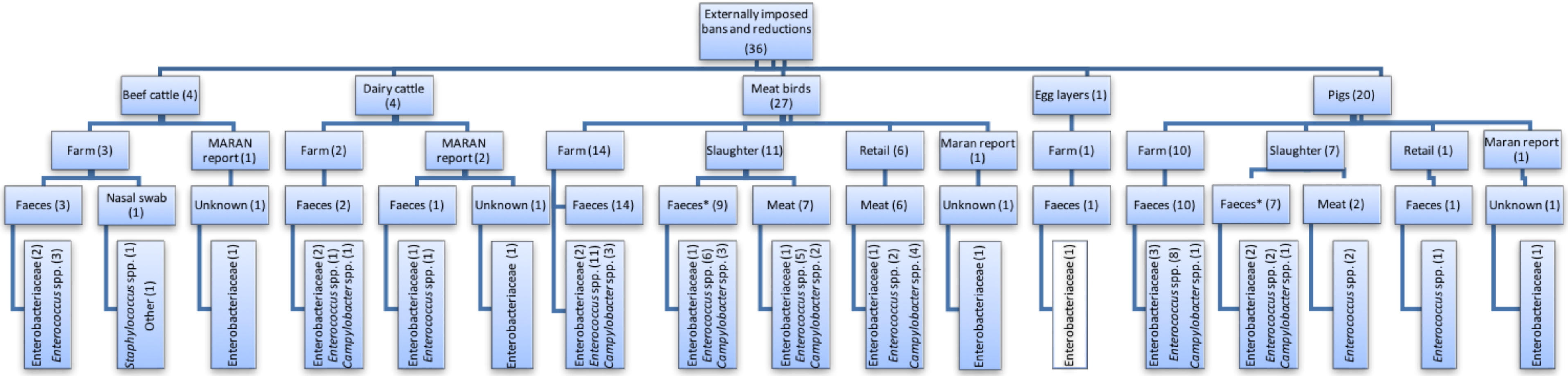
APPENDIX 3: Study characteristics flow charts

Figure 1: Flowchart depicting the species, sample point, sample type and bacteria investigated within studies where organic production systems, which reduced or eliminated antibiotic use in animals, were implemented



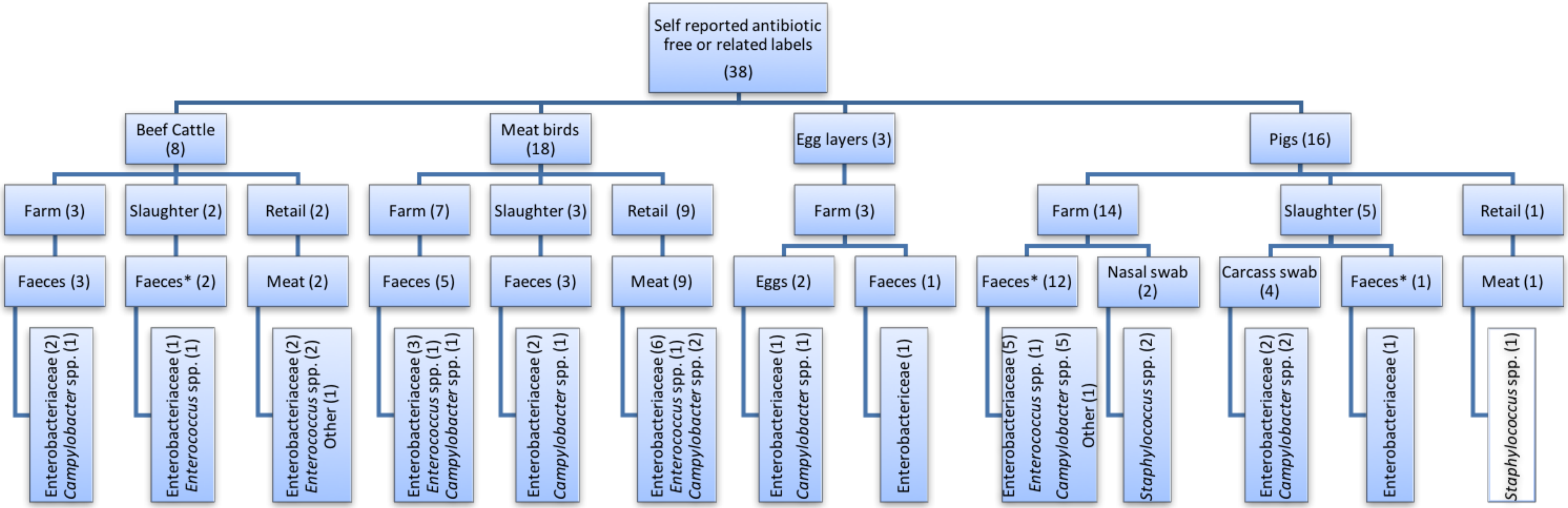
*Faeces includes caecum samples, cloacal swabs, rectal swabs, intestinal tract, colon content

Figure 2: Flowchart depicting species, sample point, sample type and bacteria investigated within studies where externally imposed bans or restrictions were placed on antibiotic use in food animals


Externally imposed bans and reductions (36)
Beef cattle (4)
Dairy cattle (4)
Meat birds (27)
Egg layers (1)
Pigs (20)
Farm (3)
MARAN report (1)
Farm (2)
MARAN report (2)
Farm (14)
Slaughter (11)
Retail (6)
Maran report (1)
Farm (1)
Farm (10)
Slaughter (7)
Retail (1)
Maran report (1)
Faeces (3)
Nasal swab (1)
Unknown (1)
Faeces (2)
Faeces (1)
Unknown (1)
Faeces (14)
Faeces* (9)
Meat (7)
Meat (6)
Unknown (1)
Faeces (1)
Faeces (10)
Faeces* (7)
Meat (2)
Faeces (1)
Unknown (1)
Enterobacteriaceae (2) *Enterococcus* spp. (3)
Staphylococcus spp. (1) Other (1)
Enterobacteriaceae (1)
Enterobacteriaceae (2) *Enterococcus* spp. (1) *Campylobacter* spp. (1)
Enterobacteriaceae (1) *Enterococcus* spp. (1)
Enterobacteriaceae (1)
Enterobacteriaceae (2) *Enterococcus* spp. (11) *Campylobacter* spp. (3)
Enterobacteriaceae (1) *Enterococcus* spp. (6) *Campylobacter* spp. (3)
Enterobacteriaceae (1) *Enterococcus* spp. (5) *Campylobacter* spp. (2)
Enterobacteriaceae (1) *Enterococcus* spp. (2) *Campylobacter* spp. (4)
Enterobacteriaceae (1)
Enterobacteriaceae (1)
Enterobacteriaceae (3) *Enterococcus* spp. (8) *Campylobacter* spp. (1)
Enterobacteriaceae (2) *Enterococcus* spp. (2) *Campylobacter* spp. (1)
Enterococcus spp. (2)
Enterococcus spp. (1)
Enterobacteriaceae (1)

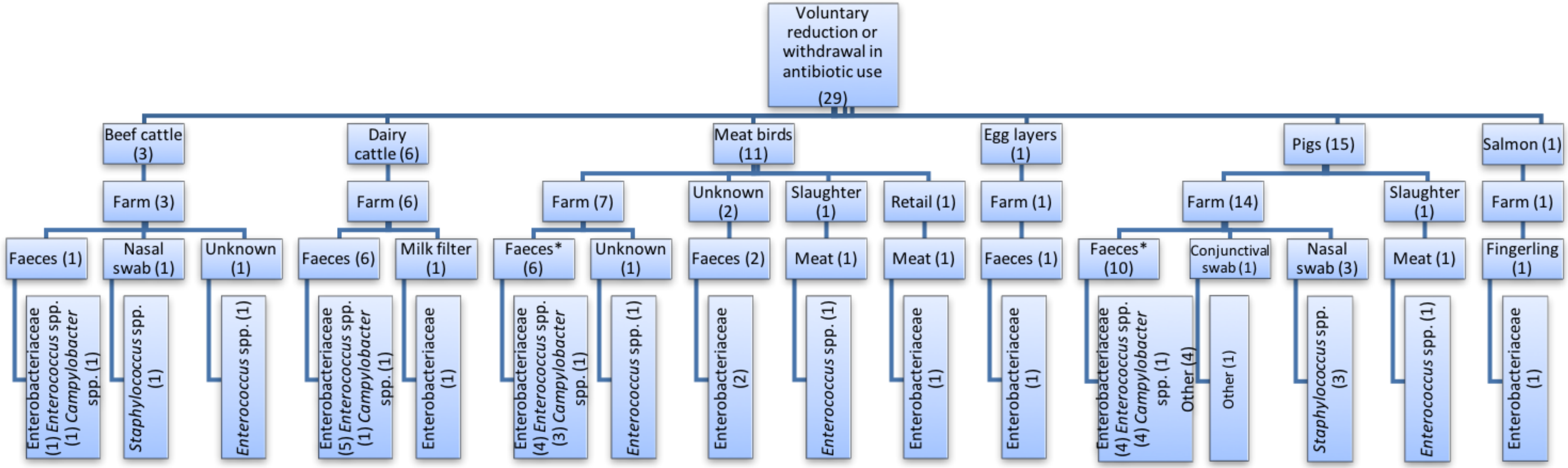
*Faeces includes caecum samples and cloacal swabs

Figure 3: Flowchart depicting the species, sample point, sample type and bacteria investigated within studies with interventions that were self-identified to be antibiotic free, raised without antibiotics, or other similar labels


Self reported antibiotic free or related labels (38)
Beef Cattle (8)
Meat birds (18)
Egg layers (3)
Pigs (16)
Farm (3)
Slaughter (2)
Retail (2)
Farm (7)
Slaughter (3)
Retail (9)
Farm (3)
Farm (14)
Slaughter (5)
Retail (1)
Faeces (3)
Faeces* (2)
Meat (2)
Faeces (5)
Faeces (3)
Meat (9)
Eggs (2)
Faeces (1)
Faeces* (12)
Nasal swab (2)
Carcass swab (4)
Faeces* (1)
Meat (1)
Enterobacteriaceae (2) *Campylobacter* spp. (1)
Enterobacteriaceae (1) *Enterococcus* spp. (1)
Enterobacteriaceae (2) *Enterococcus* spp. (2) Other (1)
Enterobacteriaceae (3) *Enterococcus* spp. (1) *Campylobacter* spp. (1)
Enterobacteriaceae (2) *Campylobacter* spp. (1)
Enterobacteriaceae (6) *Enterococcus* spp. (1) *Campylobacter* spp. (2)
Enterobacteriaceae (1) *Campylobacter* spp. (1)
Enterobactericeae (1)
Enterobacteriaceae (5) *Enterococcus* spp. (1) *Campylobacter* spp. (5) Other (1)
Staphylococcus spp. (2)
Enterobacteriaceae (2) *Campylobacter* spp. (2)
Enterobacteriaceae (1)
Staphylococcus spp. (1)

*Faeces includes rectal swabs and caecum content

Figure 4: Flowchart depicting the species, sample point, sample type and bacteria investigated in studies where there was a voluntary limitation on the use of antibiotics within production systems



*Faeces includes cloacal swabs, rectal swabs, and caecum samples

APPENDIX 4: Forest plots

Figure 1: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for Enterobacteriaceae isolates in faecal samples

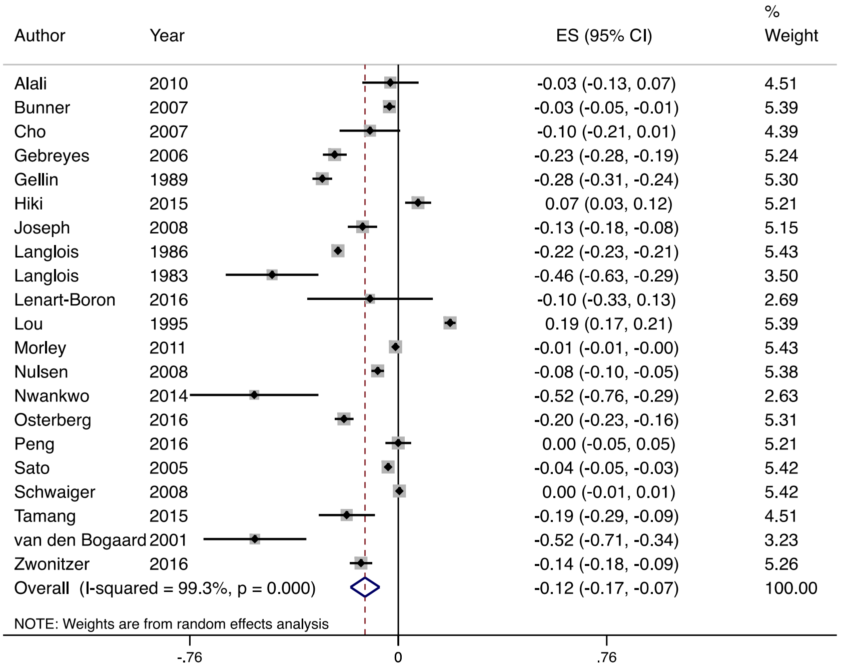


Figure 2: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for Enterobacteriaceae isolates in faecal samples

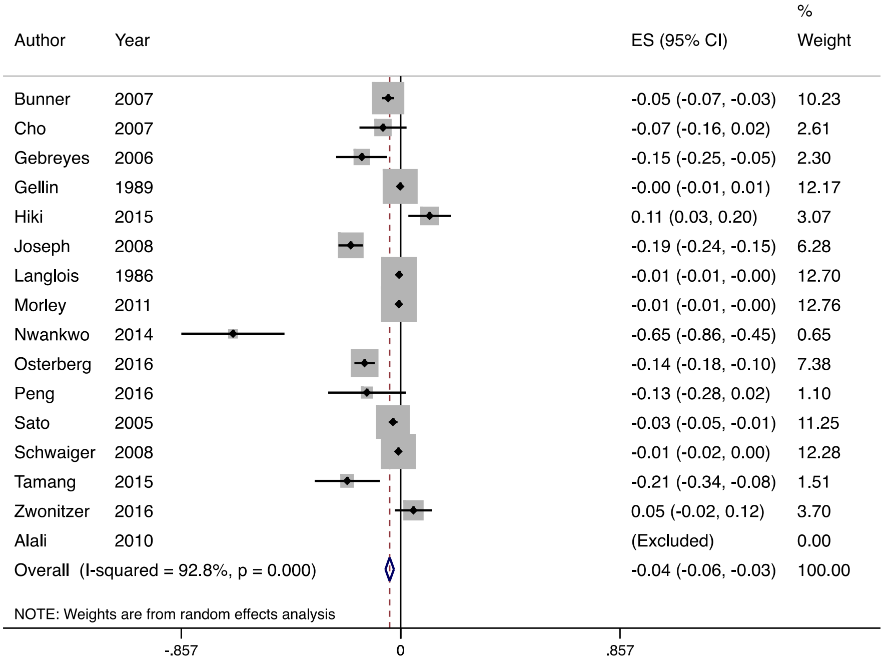
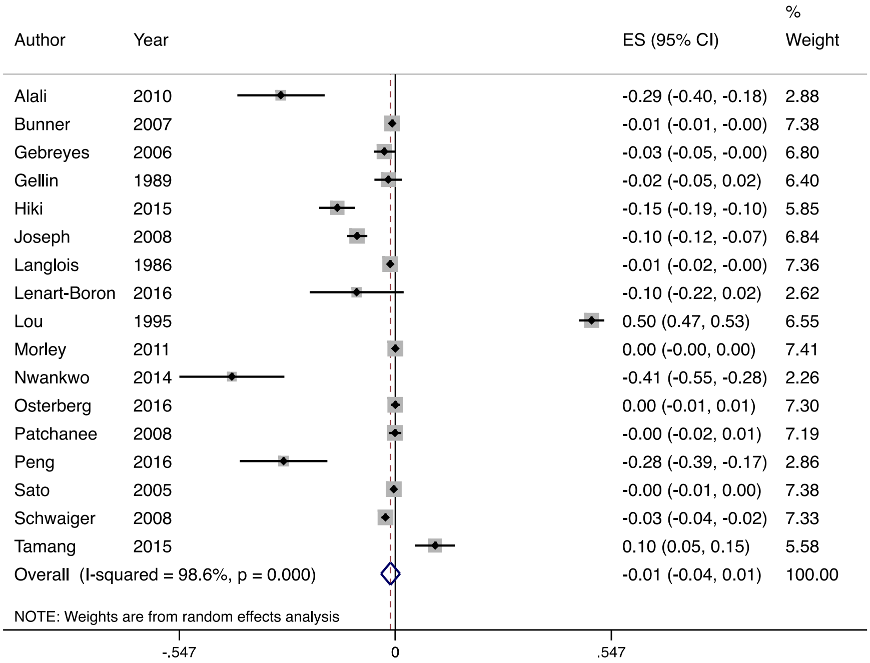


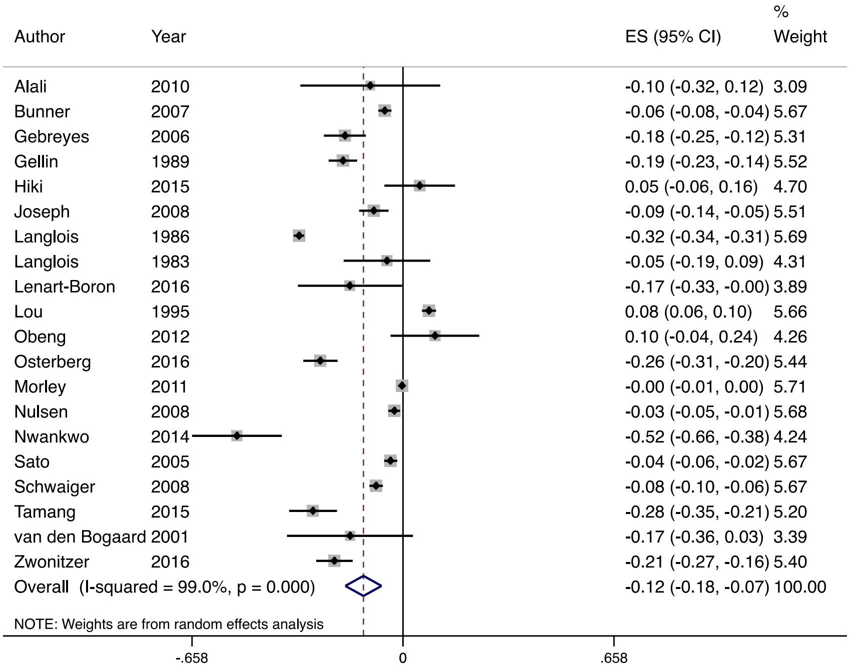
Figure 3: Forest plot of absolute risk differences of antibiotic resistance to cephalosporins for Enterobacteriaceae isolates in faecal samples



Author	Year	ES (95% CI)	% Weight
Alali	2010	-0.29 (-0.40, -0.18)	2.88
Bunner	2007	-0.01 (-0.01, -0.00)	7.38
Gebreyes	2006	-0.03 (-0.05, -0.00)	6.80
Gellin	1989	-0.02 (-0.05, 0.02)	6.40
Hiki	2015	-0.15 (-0.19, -0.10)	5.85
Joseph	2008	-0.10 (-0.12, -0.07)	6.84
Langlois	1986	-0.01 (-0.02, -0.00)	7.36
Lenart-Boron	2016	-0.10 (-0.22, 0.02)	2.62
Lou	1995	0.50 (0.47, 0.53)	6.55
Morley	2011	0.00 (-0.00, 0.00)	7.41
Nwankwo	2014	-0.41 (-0.55, -0.28)	2.26
Osterberg	2016	0.00 (-0.01, 0.01)	7.30
Patchanee	2008	-0.00 (-0.02, 0.01)	7.19
Peng	2016	-0.28 (-0.39, -0.17)	2.86
Sato	2005	-0.00 (-0.01, 0.00)	7.38
Schwaiger	2008	-0.03 (-0.04, -0.02)	7.33
Tamang	2015	0.10 (0.05, 0.15)	5.58
Overall (I-squared = 98.6%, p = 0.000)		-0.01 (-0.04, 0.01)	100.00

NOTE: Weights are from random effects analysis

Figure 4: Forest plot of absolute risk differences of antibiotic resistance to penicillins for Enterobacteriaceae isolates in faecal samples



Author	Year	ES (95% CI)	% Weight
Alali	2010	-0.10 (-0.32, 0.12)	3.09
Bunner	2007	-0.06 (-0.08, -0.04)	5.67
Gebreyes	2006	-0.18 (-0.25, -0.12)	5.31
Gellin	1989	-0.19 (-0.23, -0.14)	5.52
Hiki	2015	0.05 (-0.06, 0.16)	4.70
Joseph	2008	-0.09 (-0.14, -0.05)	5.51
Langlois	1986	-0.32 (-0.34, -0.31)	5.69
Langlois	1983	-0.05 (-0.19, 0.09)	4.31
Lenart-Boron	2016	-0.17 (-0.33, -0.00)	3.89
Lou	1995	0.08 (0.06, 0.10)	5.66
Obeng	2012	0.10 (-0.04, 0.24)	4.26
Osterberg	2016	-0.26 (-0.31, -0.20)	5.44
Morley	2011	-0.00 (-0.01, 0.00)	5.71
Nulsen	2008	-0.03 (-0.05, -0.01)	5.68
Nwankwo	2014	-0.52 (-0.66, -0.38)	4.24
Sato	2005	-0.04 (-0.06, -0.02)	5.67
Schwaiger	2008	-0.08 (-0.10, -0.06)	5.67
Tamang	2015	-0.28 (-0.35, -0.21)	5.20
van den Bogaard	2001	-0.17 (-0.36, 0.03)	3.39
Zwonitzer	2016	-0.21 (-0.27, -0.16)	5.40
Overall (I-squared = 99.0%, p = 0.000)		-0.12 (-0.18, -0.07)	100.00

NOTE: Weights are from random effects analysis

Figure 5: Forest plot of absolute risk differences of antibiotic resistance to quinolones for Enterobacteriaceae isolates in faecal samples

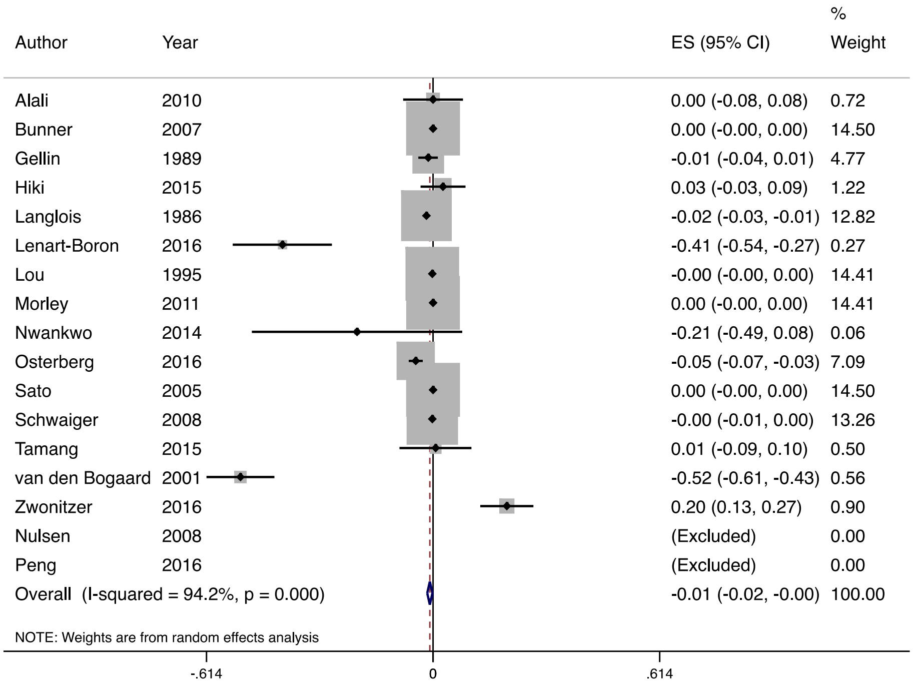


Figure 6: Forest plot of absolute risk differences of antibiotic resistance to sulfonamides for Enterobacteriaceae isolates in faecal samples

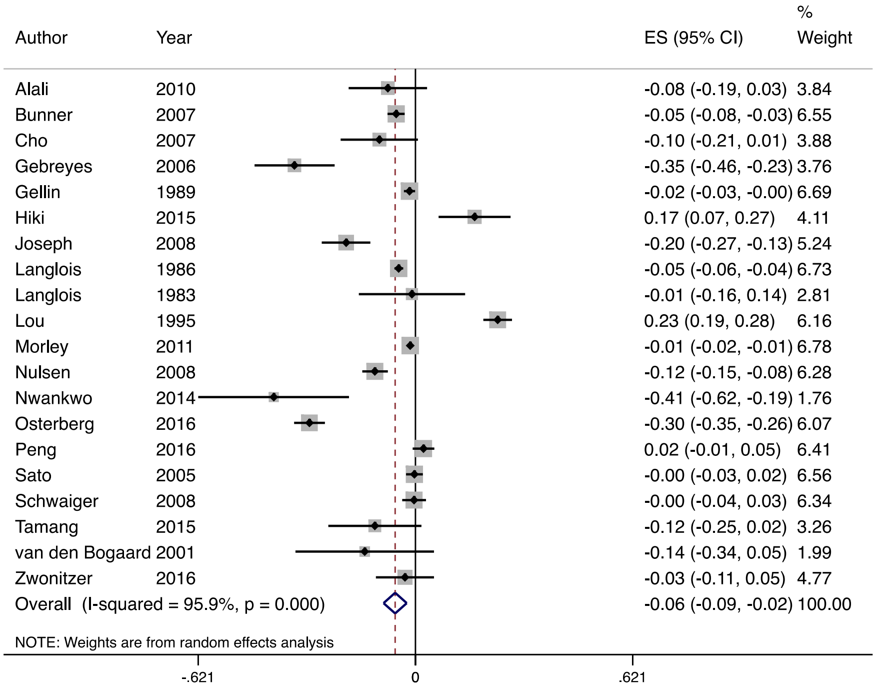


Figure 7: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for Enterobacteriaceae isolates in faecal samples

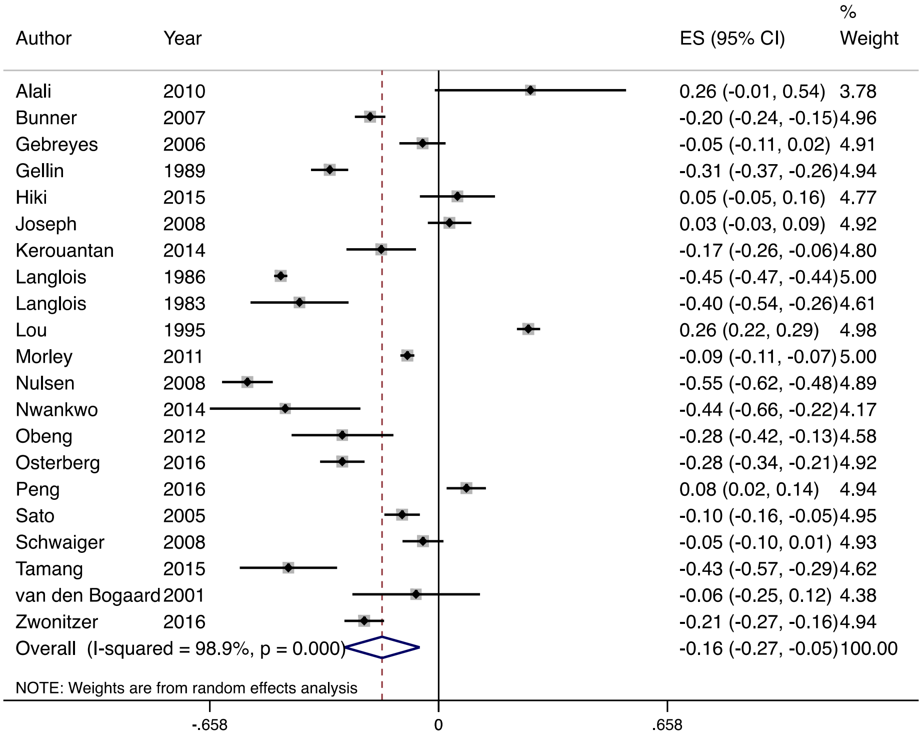


Figure 8: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for Enterobacteriaceae isolates in meat samples

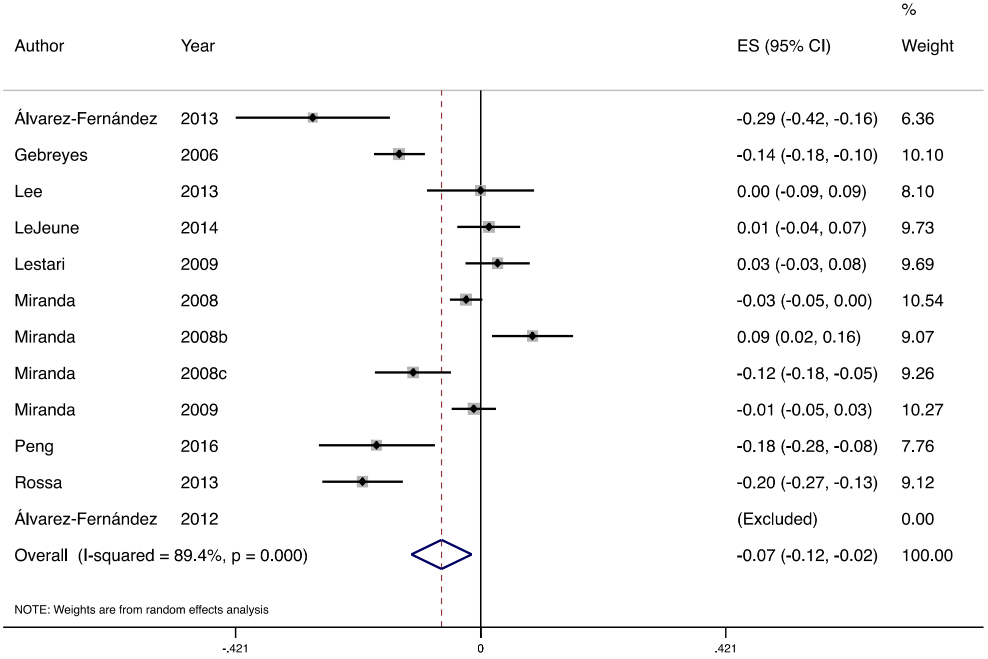


Figure 9: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for Enterobacteriaceae isolates in meat samples

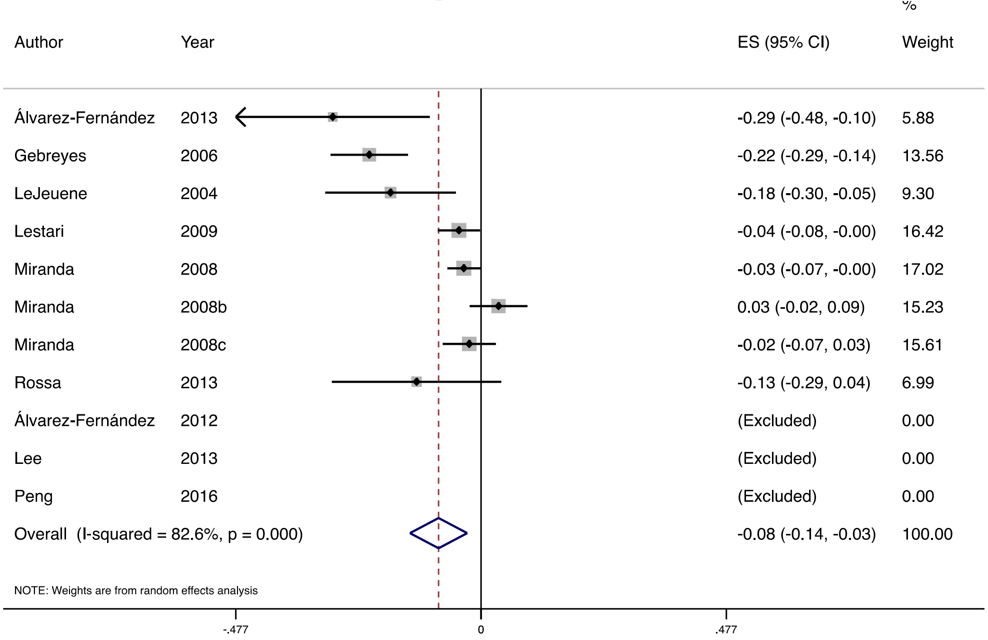


Figure 10: Forest plot of absolute risk differences of antibiotic resistance to cephalosporins for Enterobacteriaceae isolates in meat samples

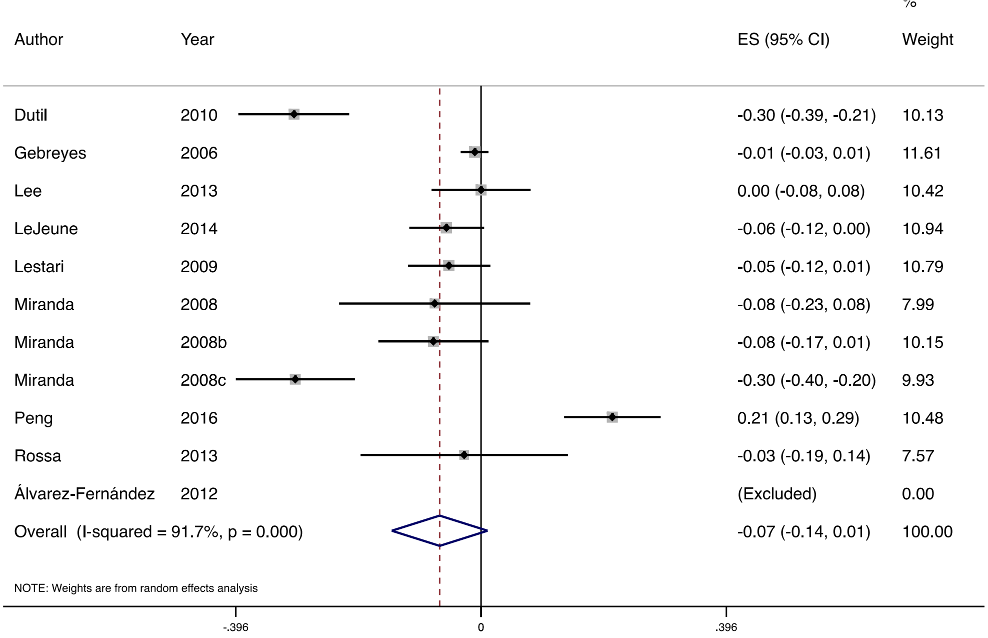


Figure 11: Forest plot of absolute risk differences of antibiotic resistance to penicillins for Enterobacteriaceae isolates in meat samples

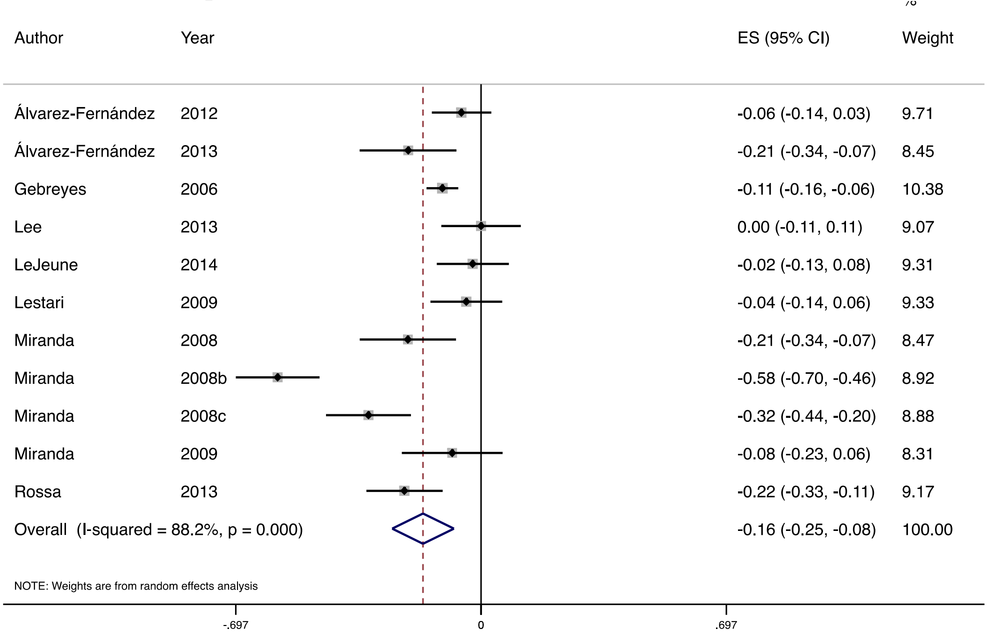


Figure 12: Forest plot of absolute risk differences of antibiotic resistance to quinolones for Enterobacteriaceae isolates in meat samples

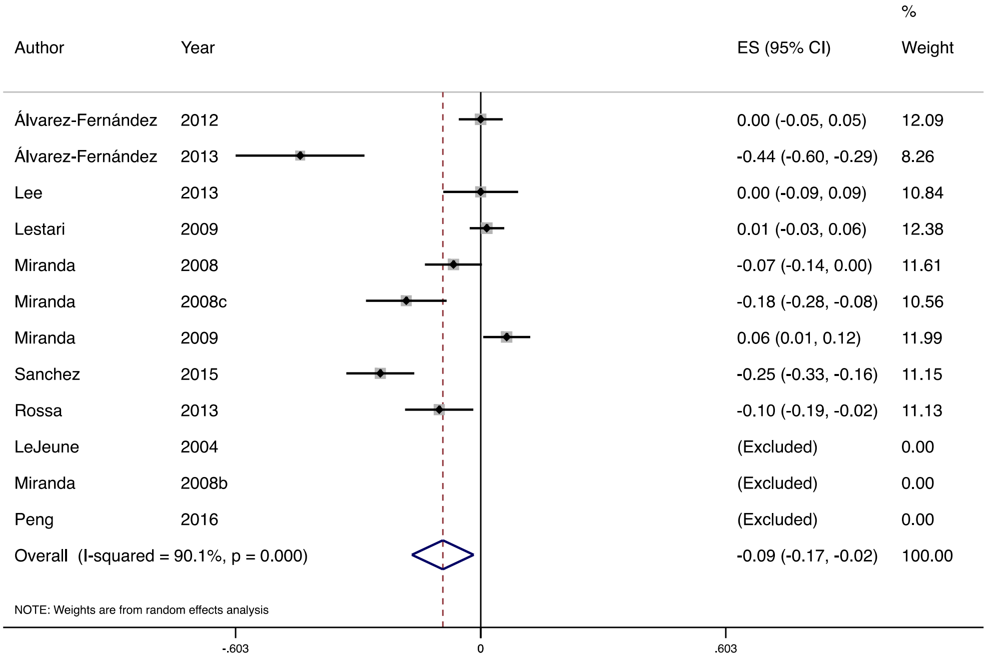


Figure 13: Forest plot of absolute risk differences of antibiotic resistance to sulfonamides for Enterobacteriaceae isolates in meat samples

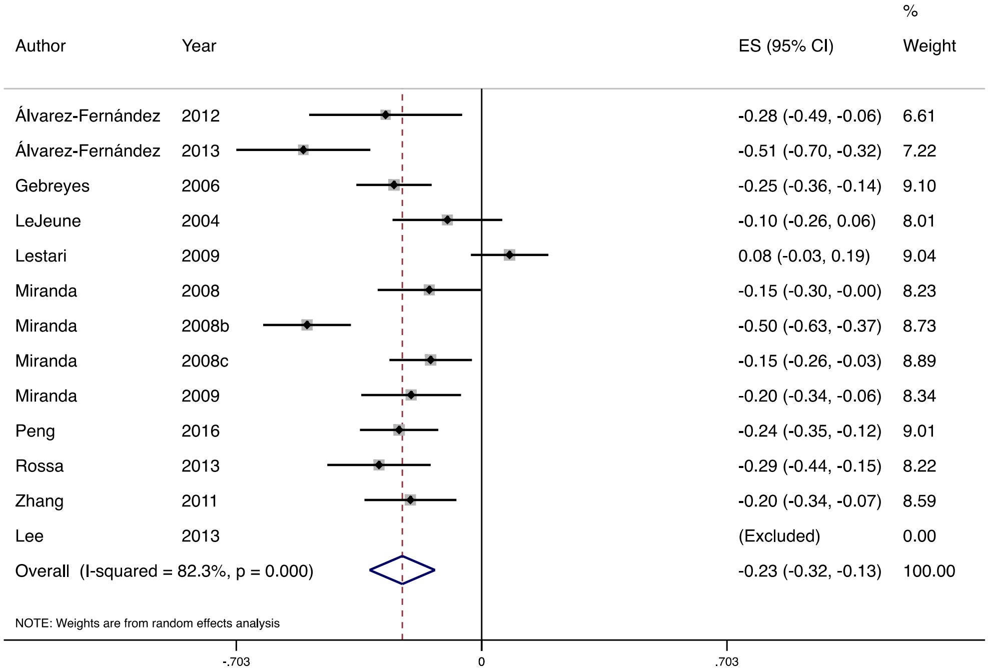


Figure 14: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for Enterobacteriaceae isolates in meat samples

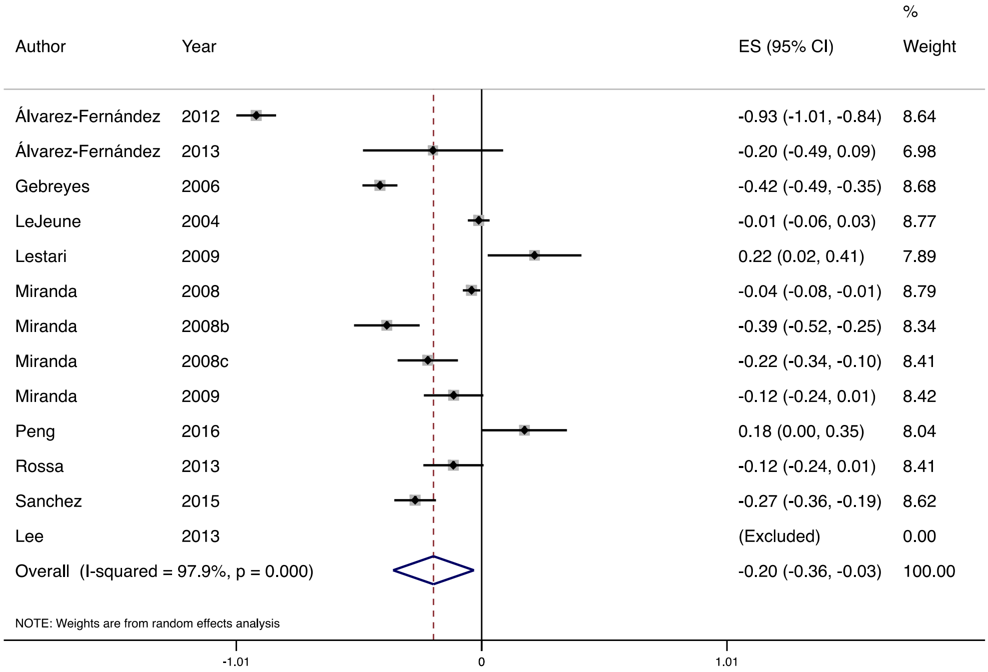


Figure 15: Forest plot of absolute risk differences in antibiotic resistance to aminoglycosides for *Enterococcus* spp. isolates in faecal samples

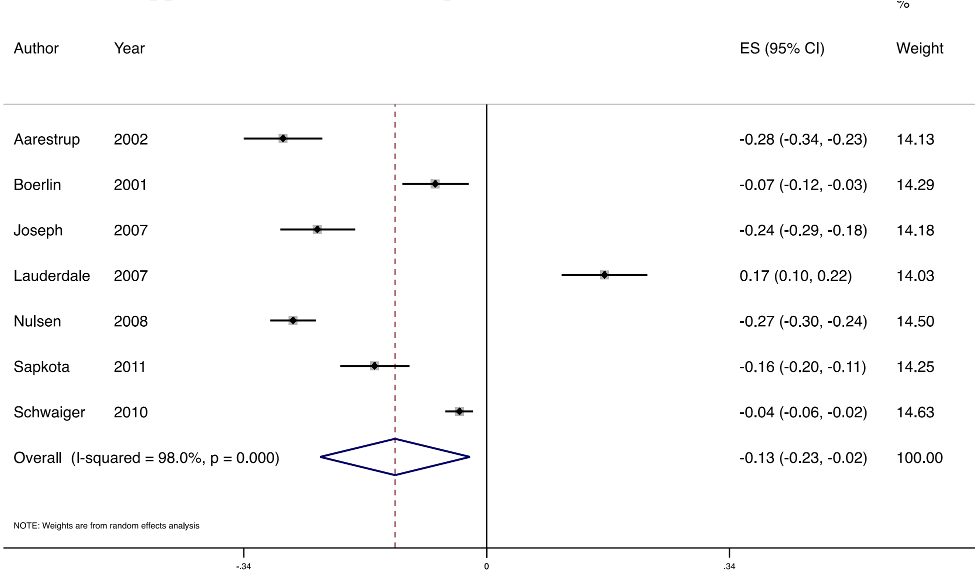


Figure 16: Forest plot of absolute risk differences in antibiotic resistance to glycopeptides for *Enterococcus* spp. isolates in faecal samples

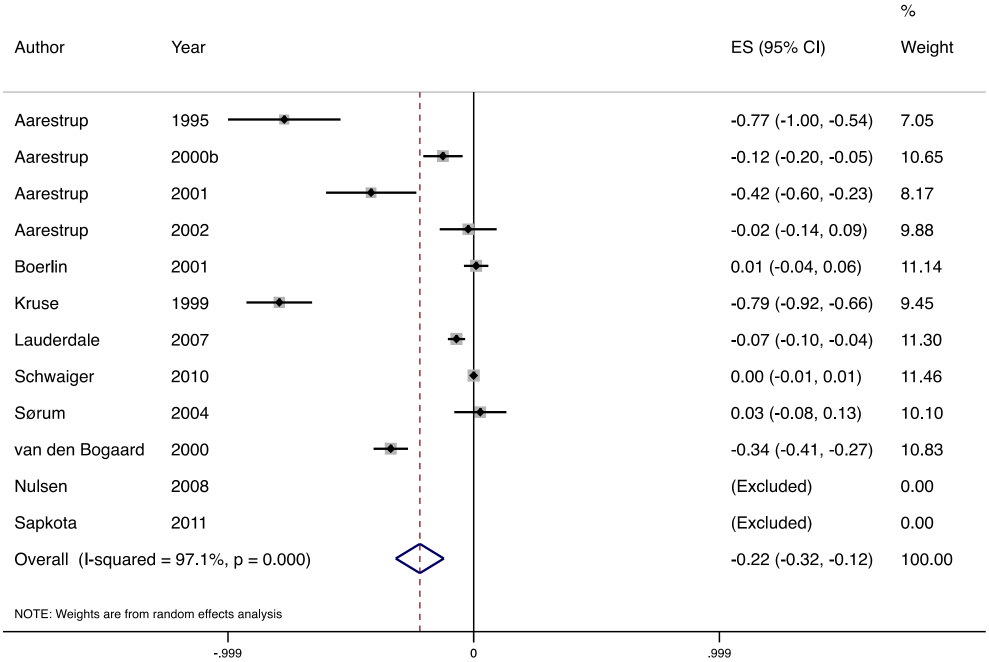


Figure 17: Forest plot of absolute risk differences in antibiotic resistance to macrolides for *Enterococcus* spp. isolates in faecal samples

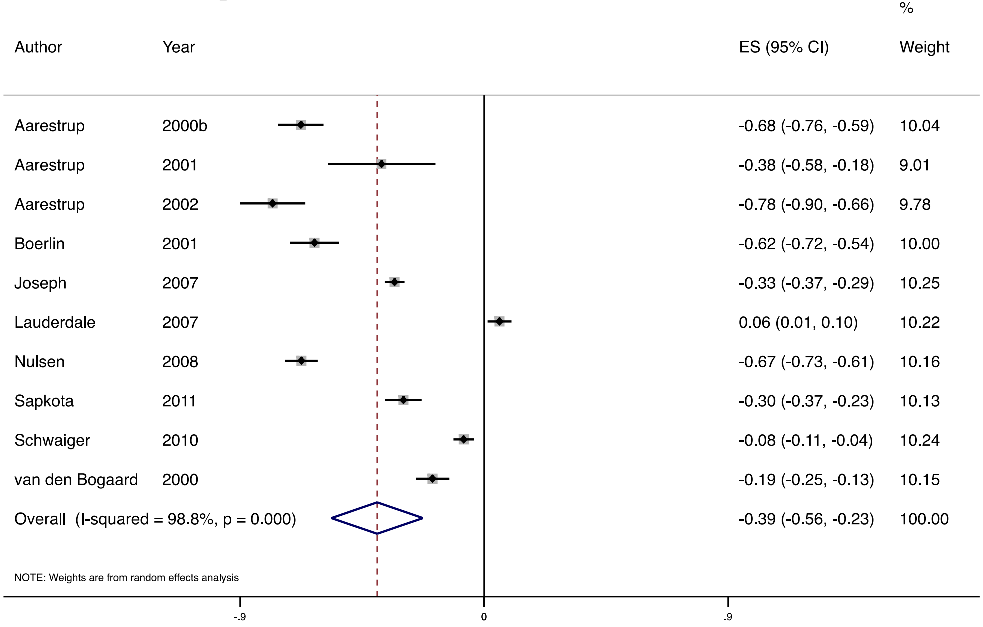


Figure 18: Forest plot of absolute risk differences in antibiotic resistance to penicillins for *Enterococcus* spp. isolates in faecal samples

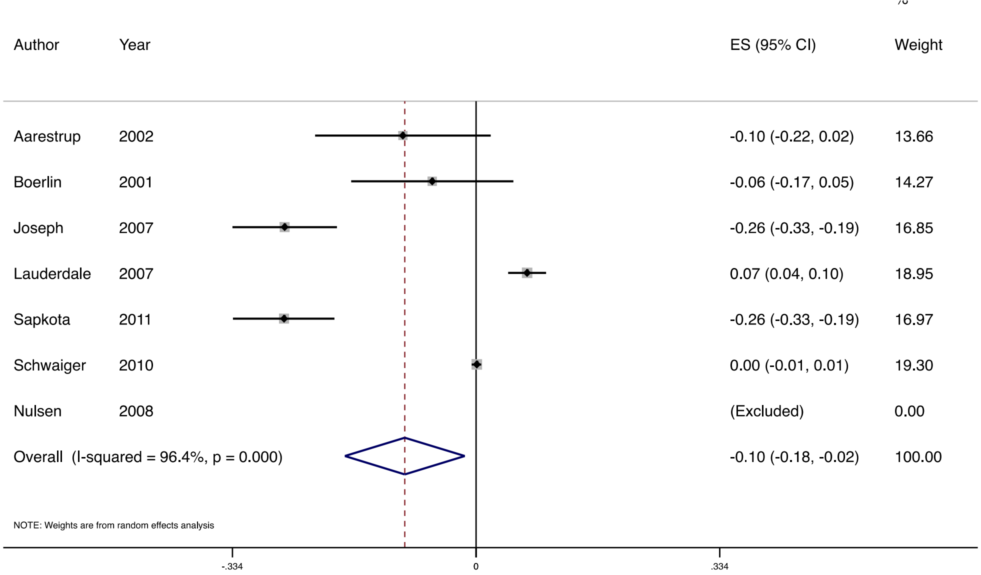


Figure 19: Forest plot of absolute risk differences in antibiotic resistance to streptogramins for *Enterococcus* spp. isolates in faecal samples

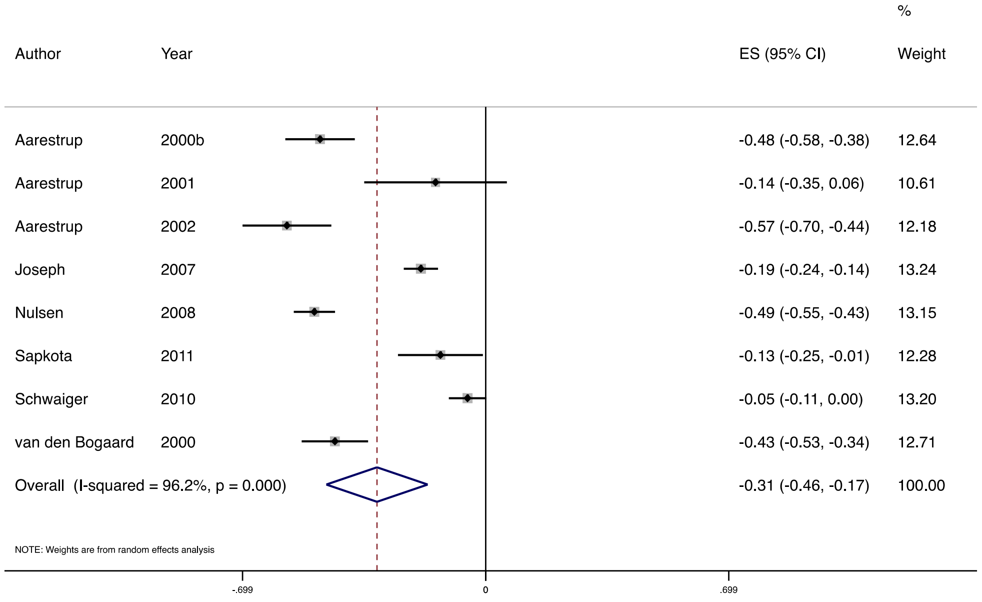


Figure 20: Forest plot of absolute risk differences in antibiotic resistance to tetracyclines for *Enterococcus* spp. isolates in faecal samples

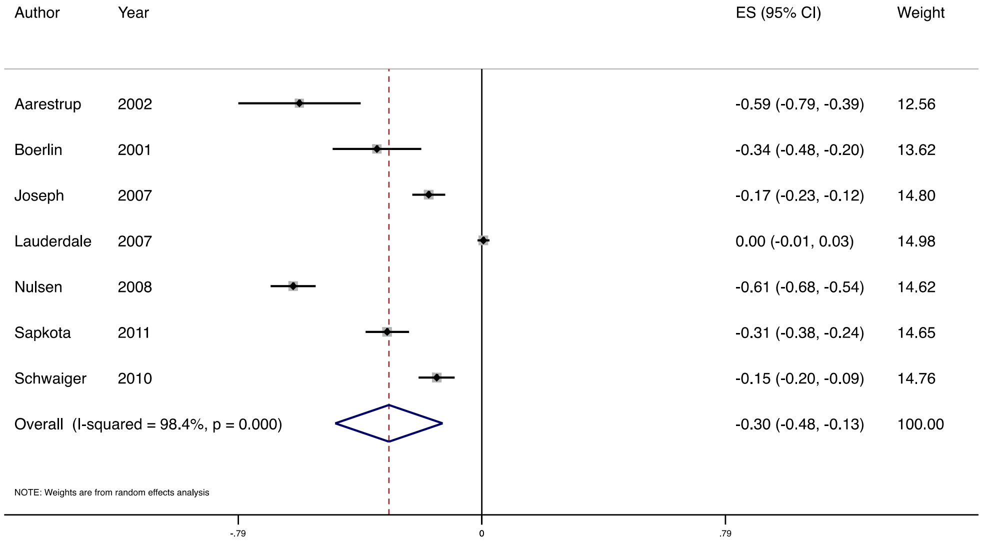


Figure 21: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for *Campylobacter* spp. isolates in faecal samples

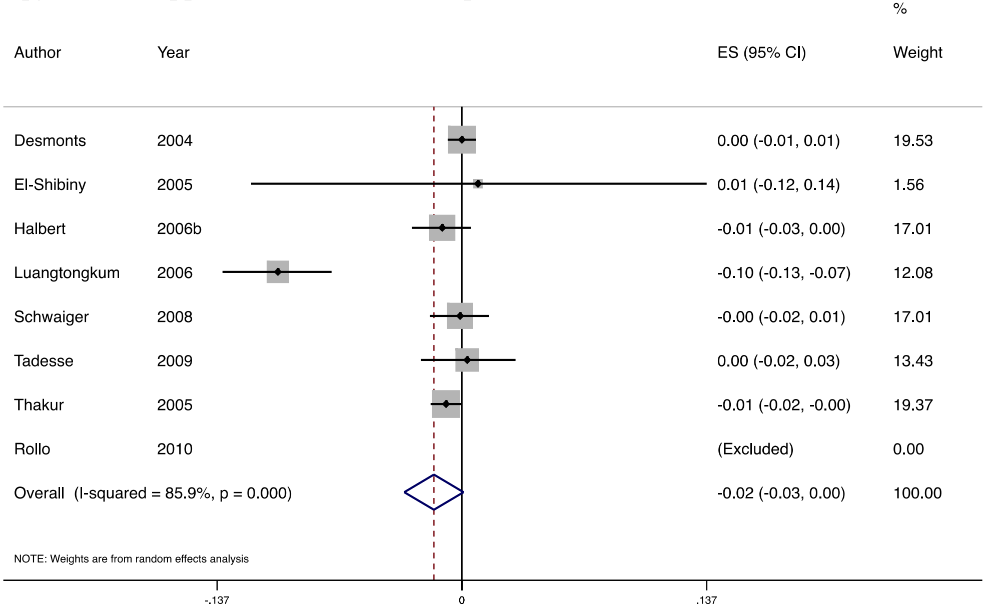


Figure 22: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for *Campylobacter* spp. isolates in faecal samples

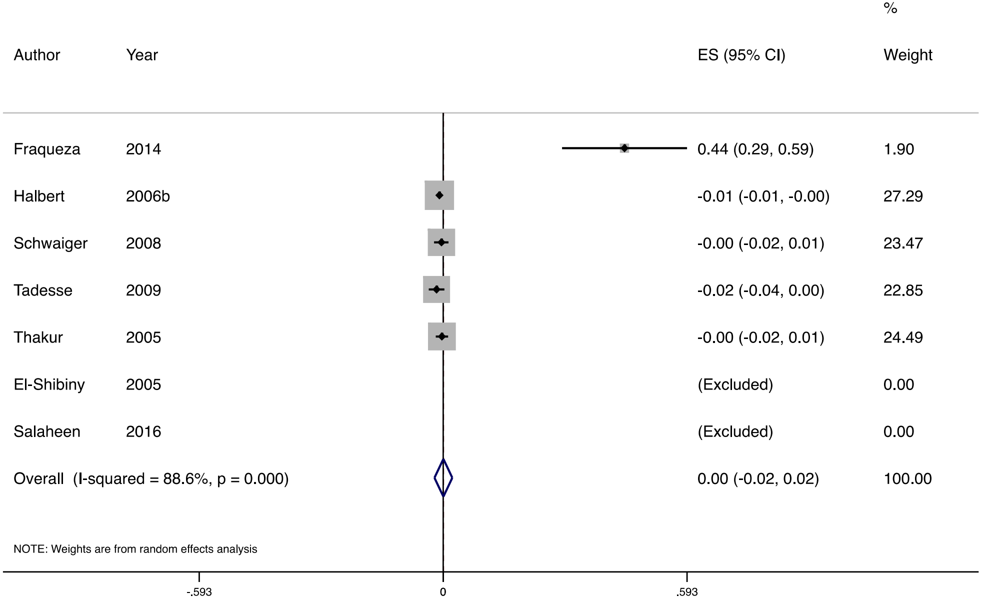


Figure 23: Forest plot of absolute risk differences of antibiotic resistance to macrolides for *Campylobacter* spp. isolates in faecal samples

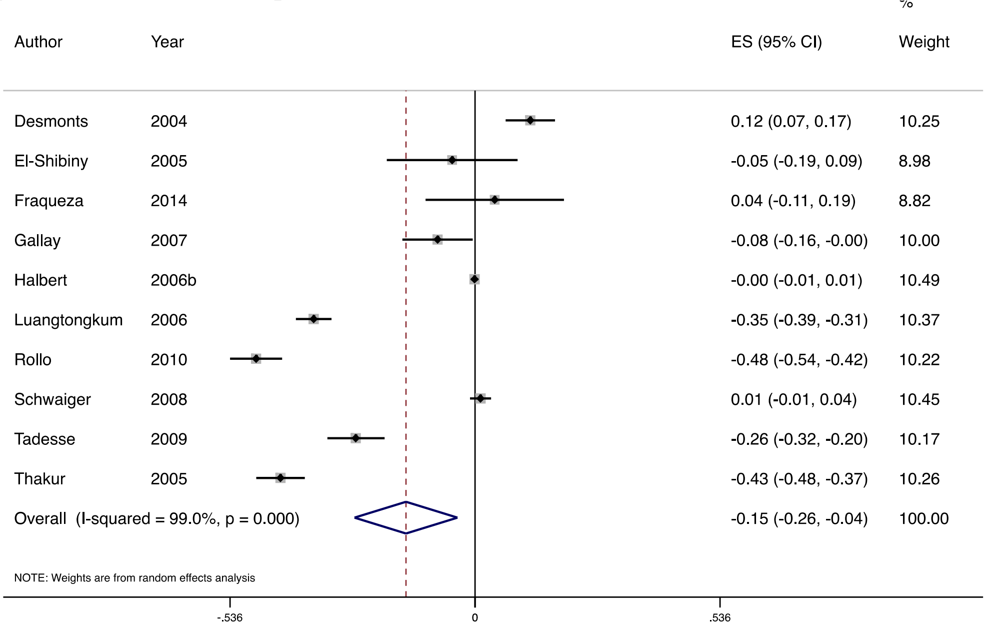


Figure 24: Forest plot of absolute risk differences of antibiotic resistance to penicillins for *Campylobacter* spp. isolates in faecal samples

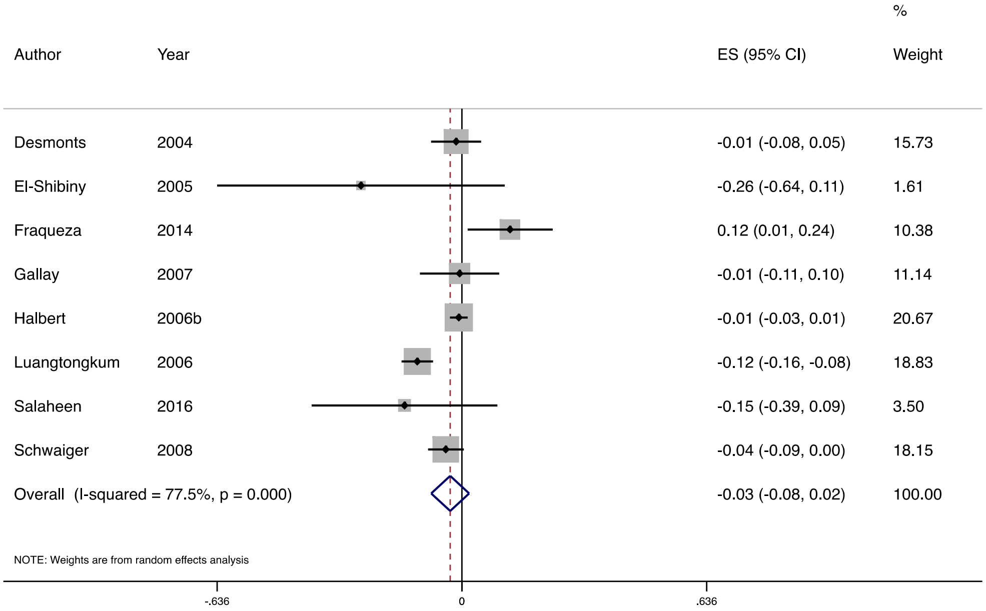


Figure 25: Forest plot of absolute risk differences of antibiotic resistance to quinolones for *Campylobacter* spp. isolates in faecal samples

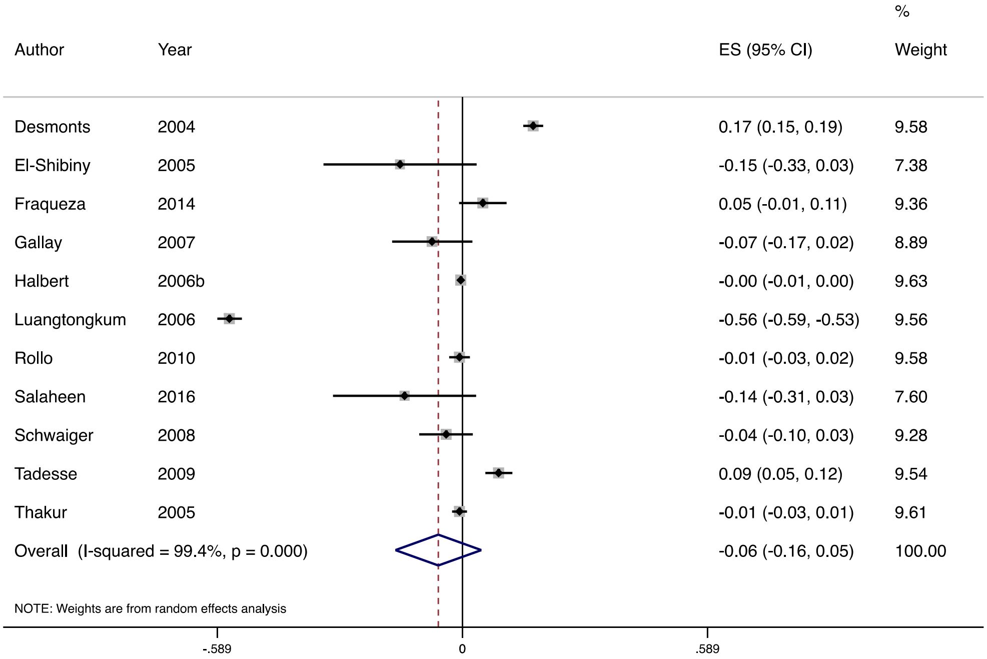


Figure 26: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for *Campylobacter* spp. isolates in faecal samples

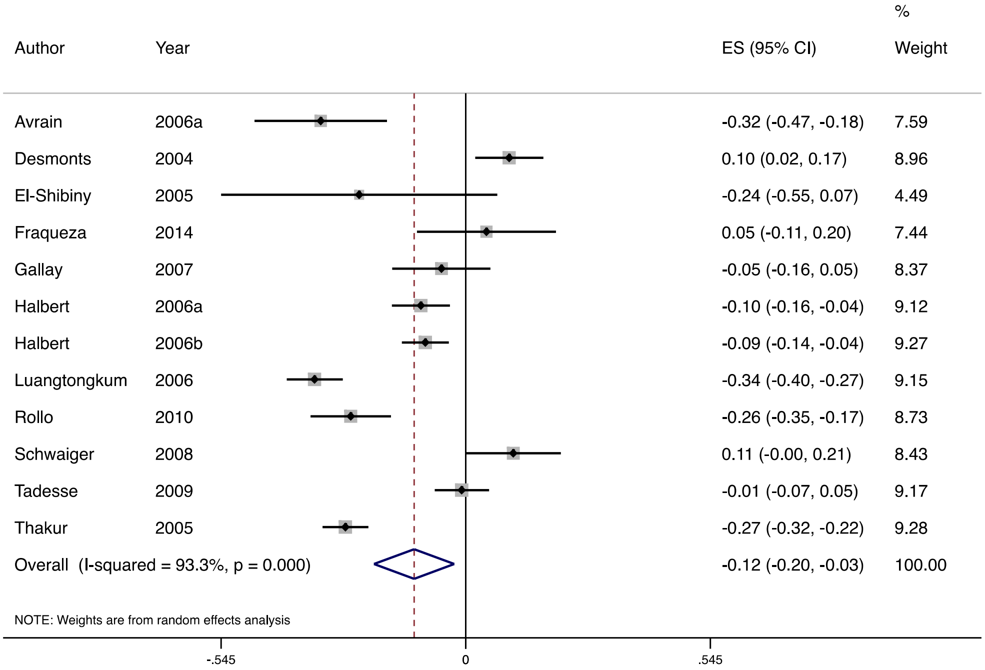


Figure 27: Forest plot of absolute risk differences of antibiotic resistance to macrolides for *Campylobacter* spp. isolates in meat samples

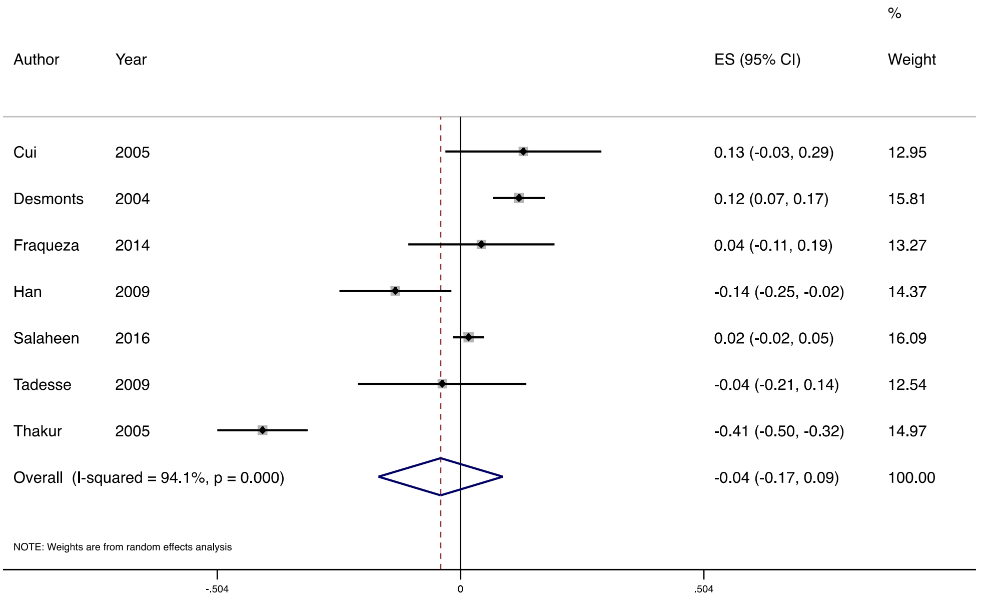


Figure 28: Forest plot of absolute risk differences of antibiotic resistance to quinolones for *Campylobacter* spp. isolates in meat samples

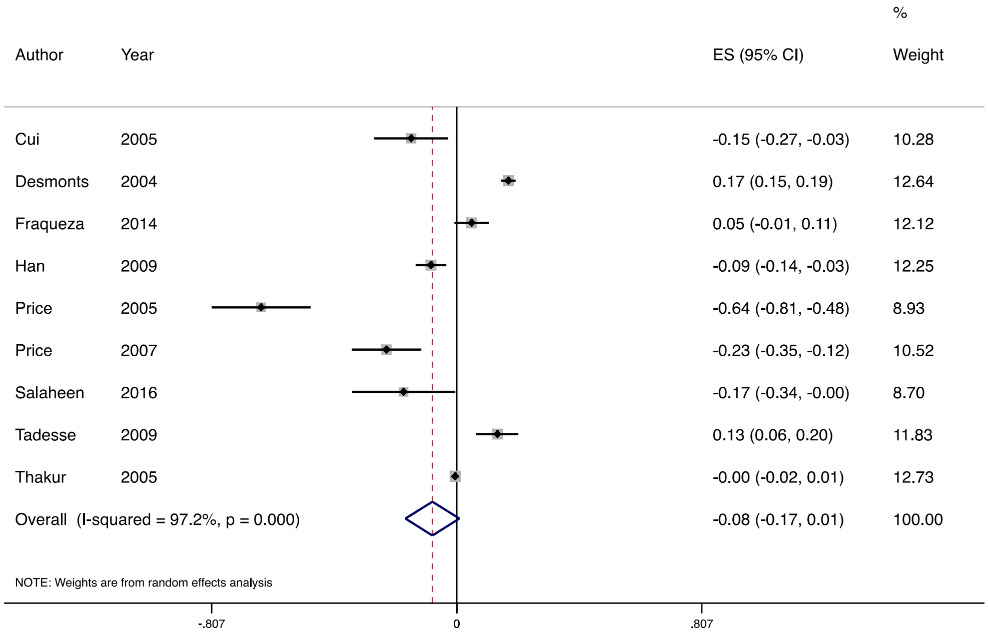


Figure 29: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for *Campylobacter* spp. isolates in meat samples

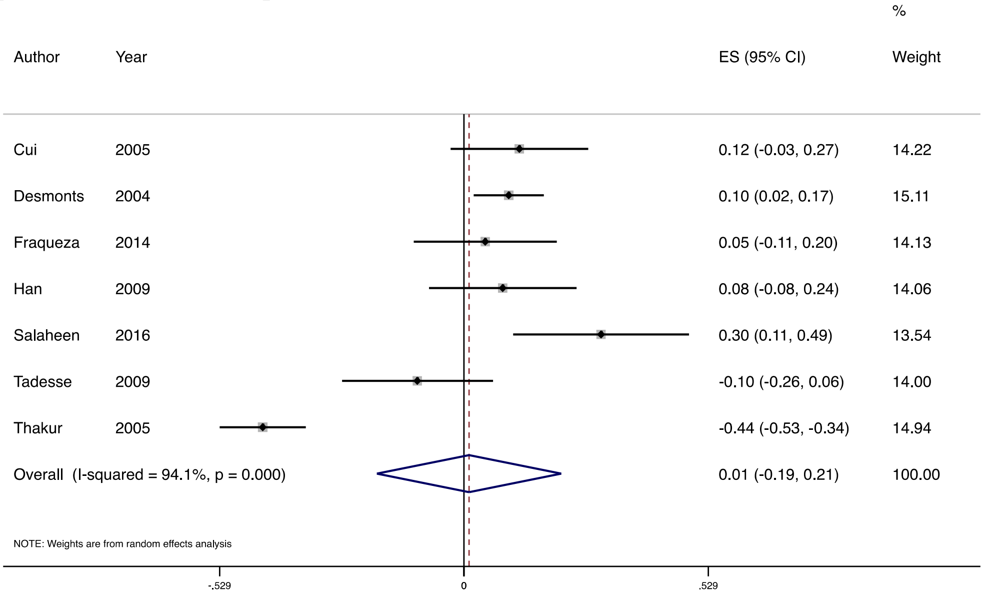


Figure 30: Forest plot of absolute risk differences in antibiotic resistance to aminoglycosides for *Staphylococcus* spp. isolates in milk samples

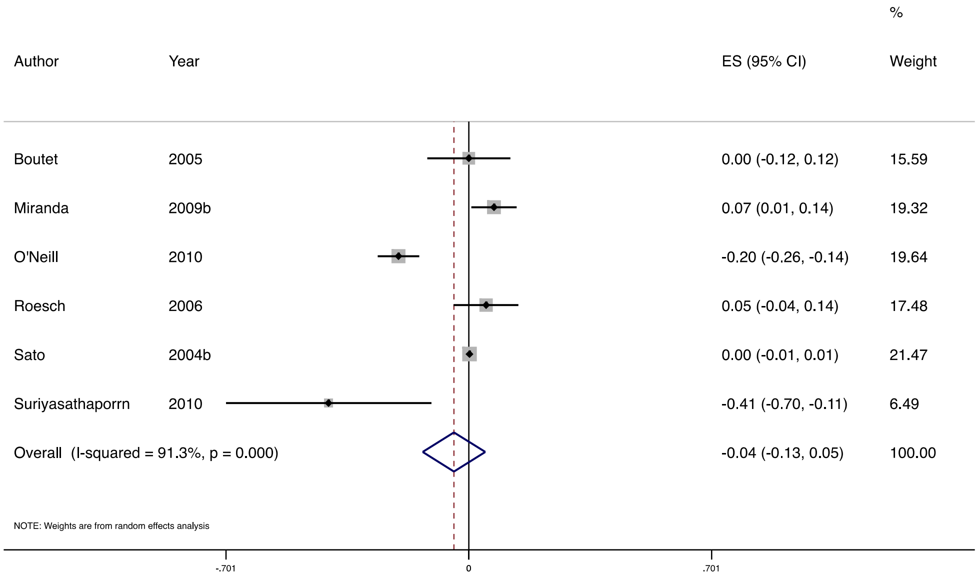


Figure 31: Forest plot of absolute risk differences in antibiotic resistance to lincosamides for *Staphylococcus* spp. isolates in milk samples

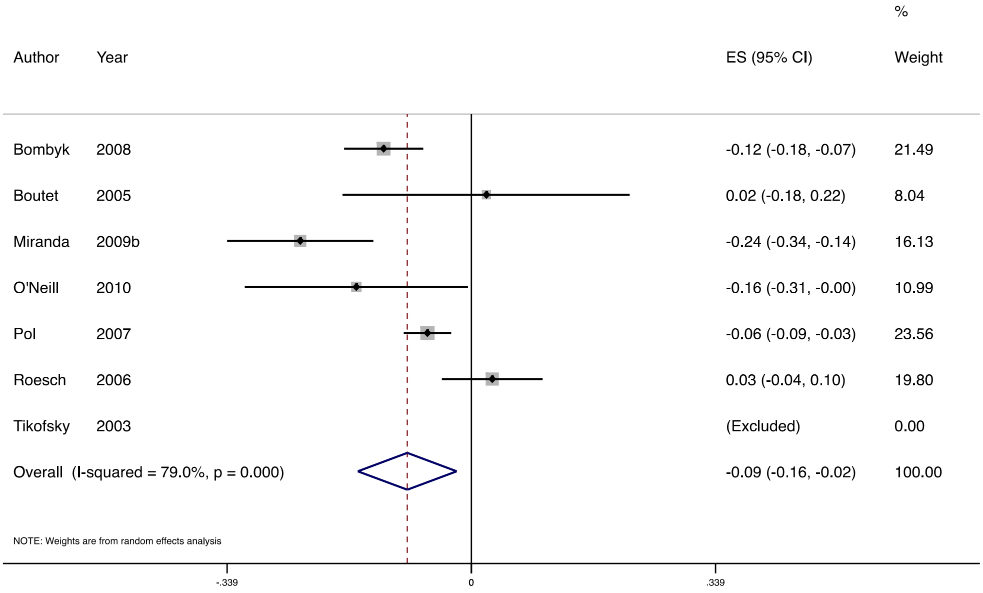


Figure 32: Forest plot of absolute risk differences in antibiotic resistance to macrolides for *Staphylococcus* spp. isolates in milk samples

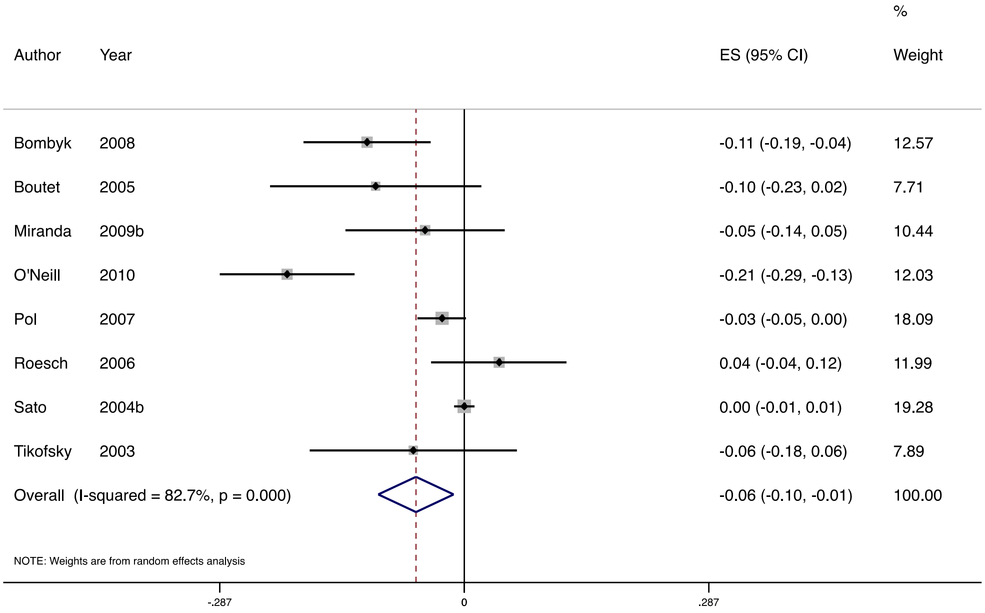


Figure 33: Forest plot of absolute risk differences in antibiotic resistance to penicillins for *Staphylococcus* spp. isolates in milk samples

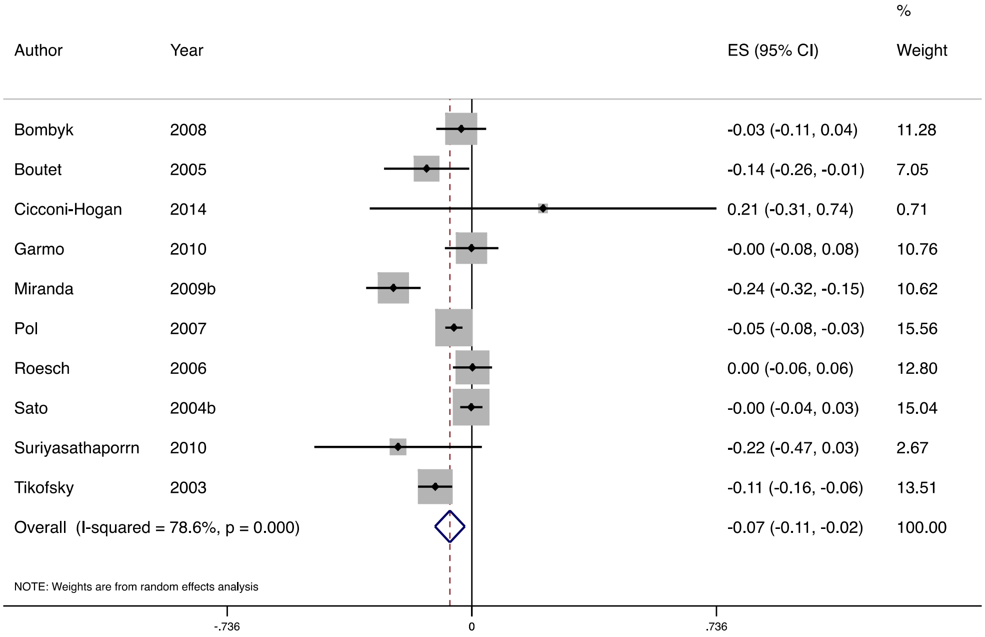


Figure 34: Forest plot of absolute risk differences in antibiotic resistance to sulfonamides for *Staphylococcus* spp. isolates in milk samples

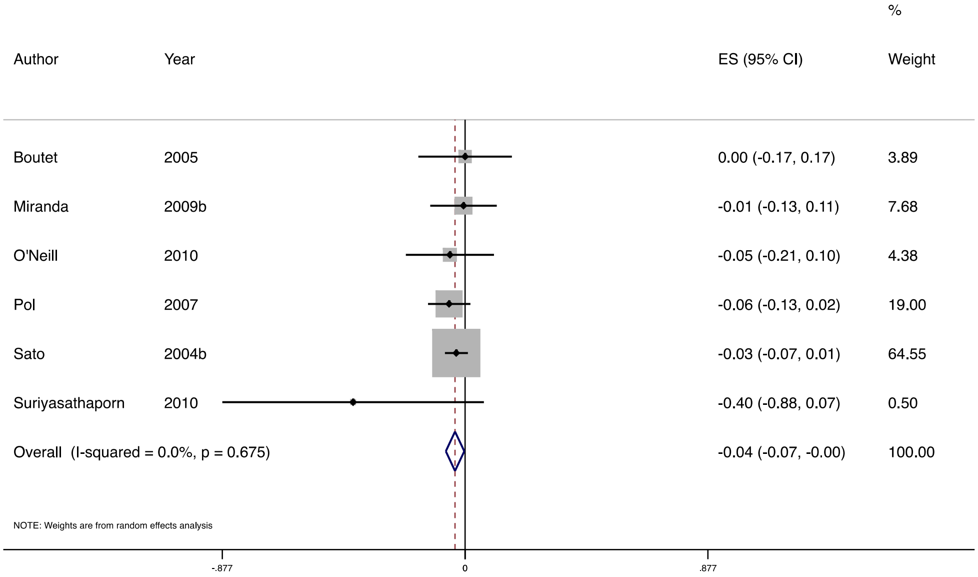
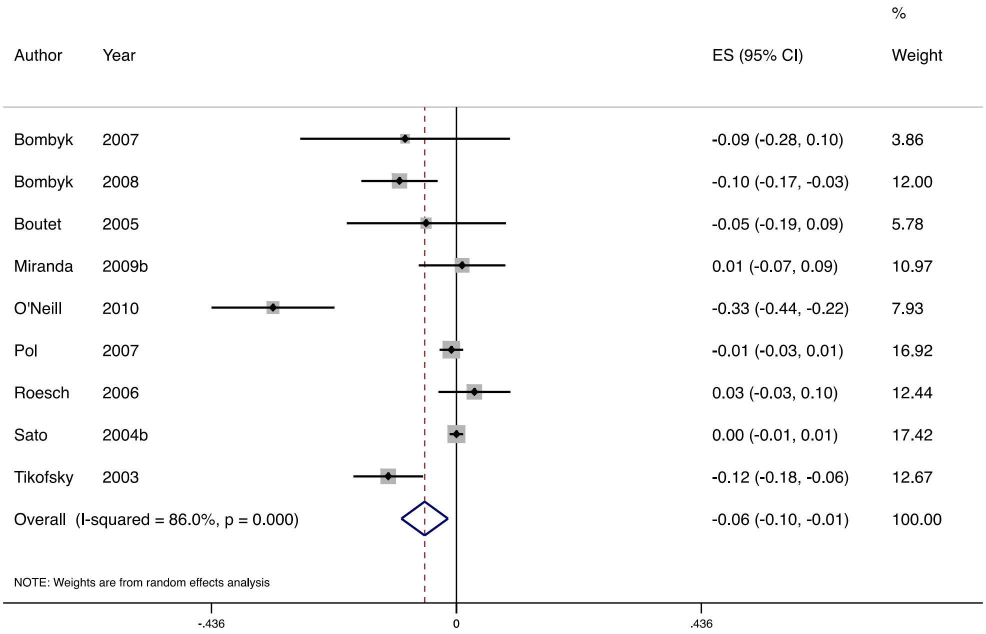


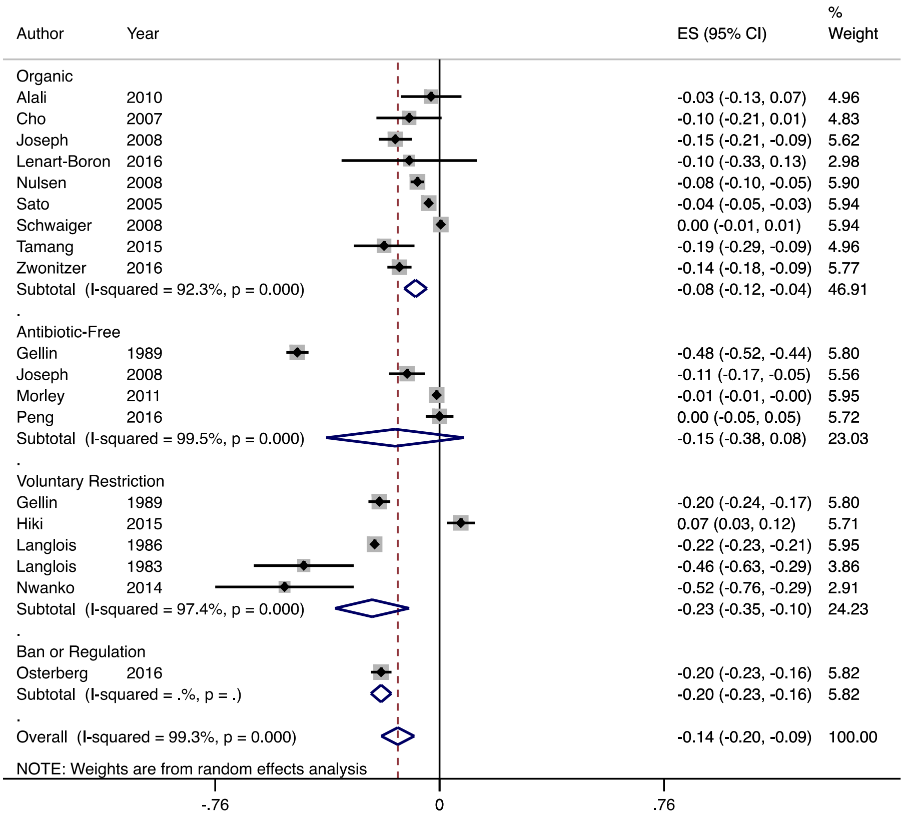
Figure 35: Forest plot of absolute risk differences in antibiotic resistance to tetracyclines for *Staphylococcus* spp. isolates in milk samples



Author	Year	ES (95% CI)	% Weight
Bombyk	2007	-0.09 (-0.28, 0.10)	3.86
Bombyk	2008	-0.10 (-0.17, -0.03)	12.00
Boutet	2005	-0.05 (-0.19, 0.09)	5.78
Miranda	2009b	0.01 (-0.07, 0.09)	10.97
O'Neill	2010	-0.33 (-0.44, -0.22)	7.93
Pol	2007	-0.01 (-0.03, 0.01)	16.92
Roesch	2006	0.03 (-0.03, 0.10)	12.44
Sato	2004b	0.00 (-0.01, 0.01)	17.42
Tikofsky	2003	-0.12 (-0.18, -0.06)	12.67
Overall (I-squared = 86.0%, p = 0.000)		-0.06 (-0.10, -0.01)	100.00

NOTE: Weights are from random effects analysis

Figure 36: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for Enterobacteriaceae isolates in faecal samples, stratified by intervention



Author	Year	ES (95% CI)	% Weight
Organic			
Alali	2010	-0.03 (-0.13, 0.07)	4.96
Cho	2007	-0.10 (-0.21, 0.01)	4.83
Joseph	2008	-0.15 (-0.21, -0.09)	5.62
Lenart-Boron	2016	-0.10 (-0.33, 0.13)	2.98
Nulsen	2008	-0.08 (-0.10, -0.05)	5.90
Sato	2005	-0.04 (-0.05, -0.03)	5.94
Schwaiger	2008	0.00 (-0.01, 0.01)	5.94
Tamang	2015	-0.19 (-0.29, -0.09)	4.96
Zwonitzer	2016	-0.14 (-0.18, -0.09)	5.77
Subtotal (I-squared = 92.3%, p = 0.000)		-0.08 (-0.12, -0.04)	46.91
.			
Antibiotic-Free			
Gellin	1989	-0.48 (-0.52, -0.44)	5.80
Joseph	2008	-0.11 (-0.17, -0.05)	5.56
Morley	2011	-0.01 (-0.01, -0.00)	5.95
Peng	2016	0.00 (-0.05, 0.05)	5.72
Subtotal (I-squared = 99.5%, p = 0.000)		-0.15 (-0.38, 0.08)	23.03
.			
Voluntary Restriction			
Gellin	1989	-0.20 (-0.24, -0.17)	5.80
Hiki	2015	0.07 (0.03, 0.12)	5.71
Langlois	1986	-0.22 (-0.23, -0.21)	5.95
Langlois	1983	-0.46 (-0.63, -0.29)	3.86
Nwanko	2014	-0.52 (-0.76, -0.29)	2.91
Subtotal (I-squared = 97.4%, p = 0.000)		-0.23 (-0.35, -0.10)	24.23
.			
Ban or Regulation			
Osterberg	2016	-0.20 (-0.23, -0.16)	5.82
Subtotal (I-squared = .%, p = .)		-0.20 (-0.23, -0.16)	5.82
.			
Overall (I-squared = 99.3%, p = 0.000)		-0.14 (-0.20, -0.09)	100.00

NOTE: Weights are from random effects analysis

Figure 37: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for Enterobacteriaceae isolates in faecal samples, stratified by intervention type

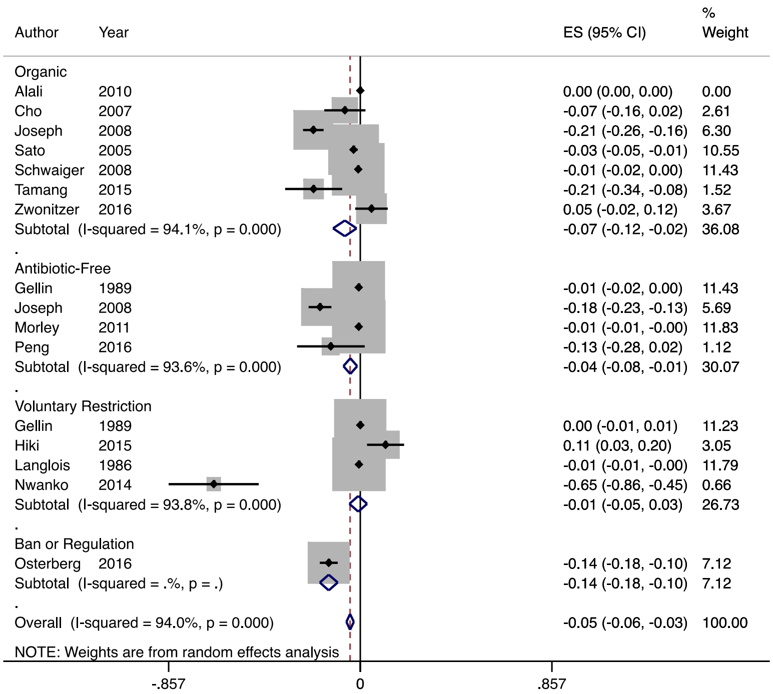


Figure 38: Forest plot of absolute risk differences of antibiotic resistance to cephalosporins for Enterobacteriaceae isolates in faecal samples, stratified by intervention

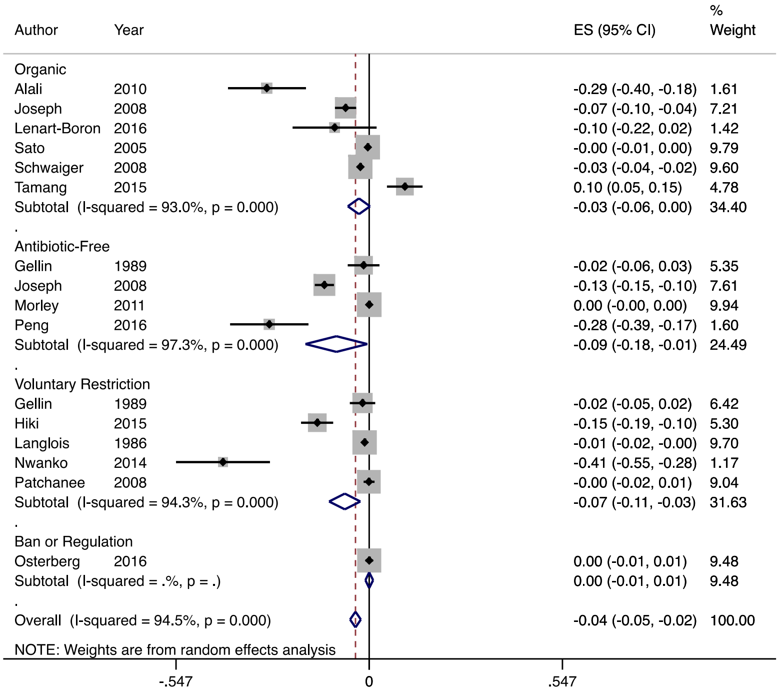


Figure 39: Forest plot of absolute risk differences of antibiotic resistance to penicillins for Enterobacteriaceae isolates in faecal samples, stratified by intervention

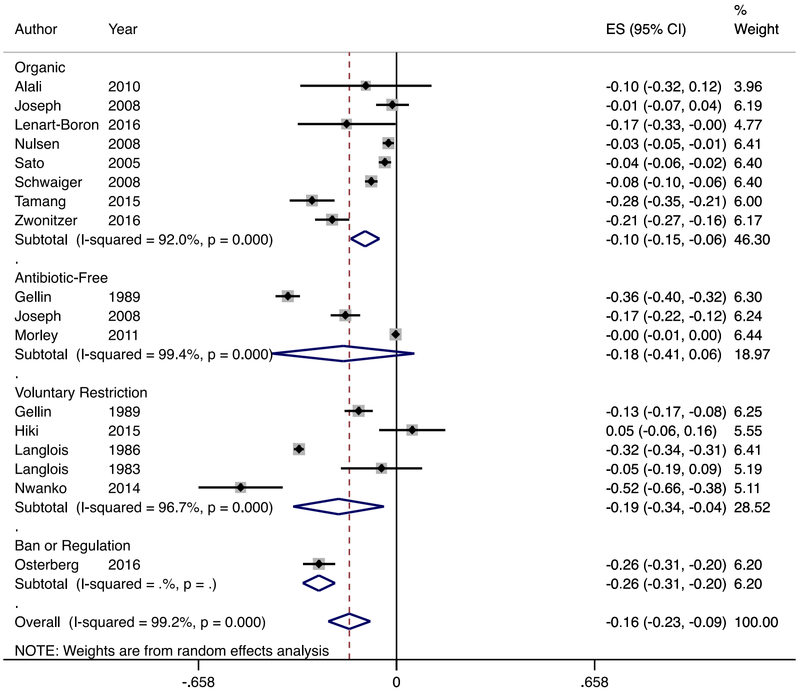


Figure 40: Forest plot of absolute risk differences of antibiotic resistance to quinolones for Enterobacteriaceae isolates in faecal samples, stratified by intervention

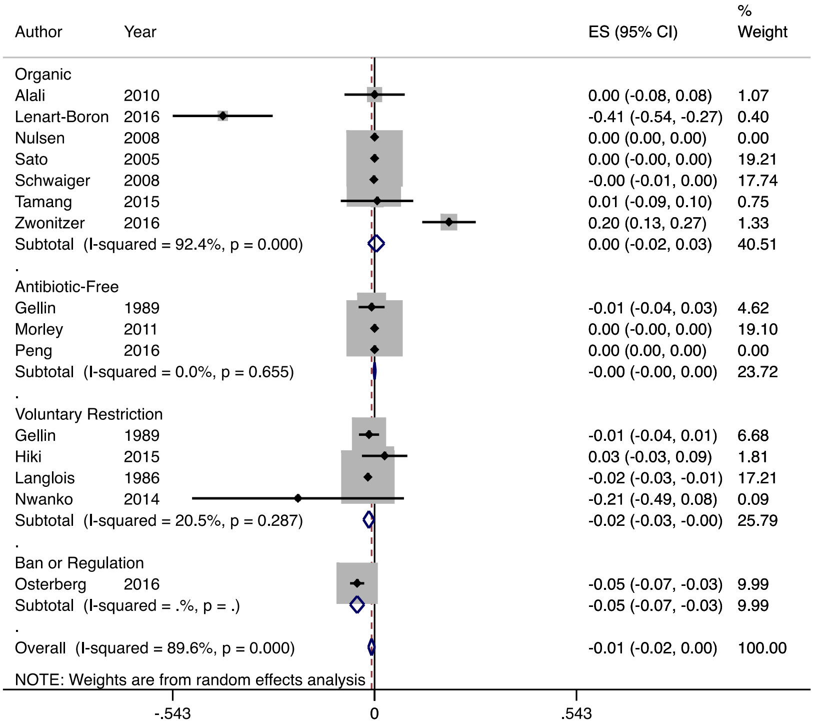


Figure 41: Forest plot of absolute risk differences of antibiotic resistance to sulfonamides for Enterobacteriaceae isolates in faecal samples, stratified by intervention type

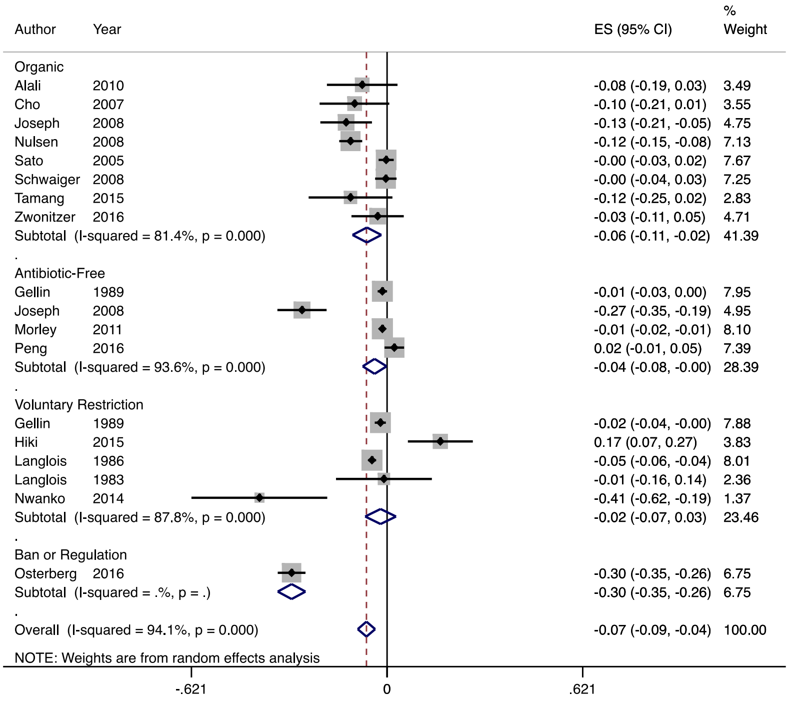


Figure 42: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for Enterobacteriaceae isolates in faecal samples, stratified by intervention type

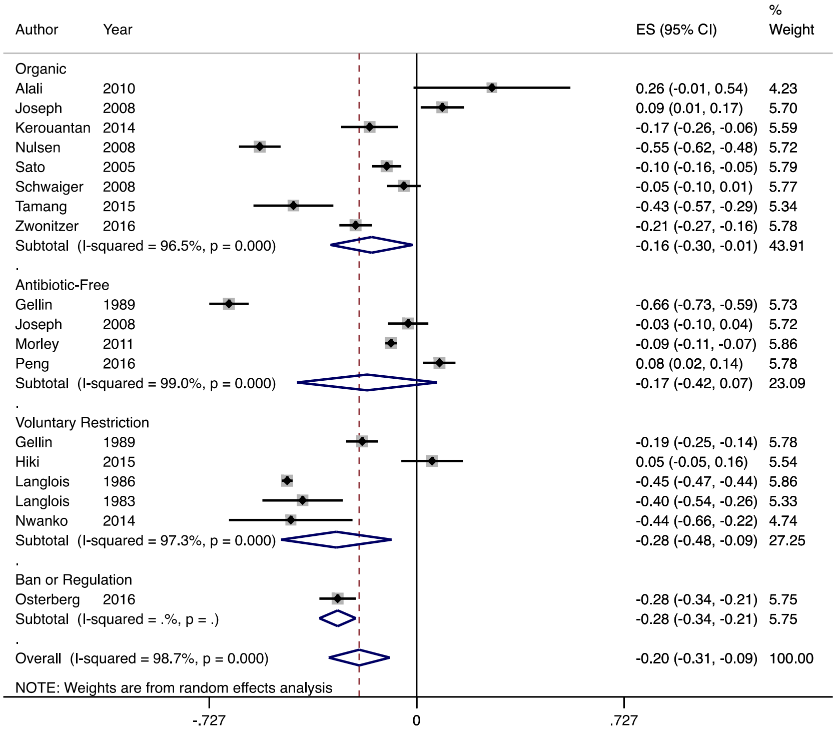


Figure 43: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

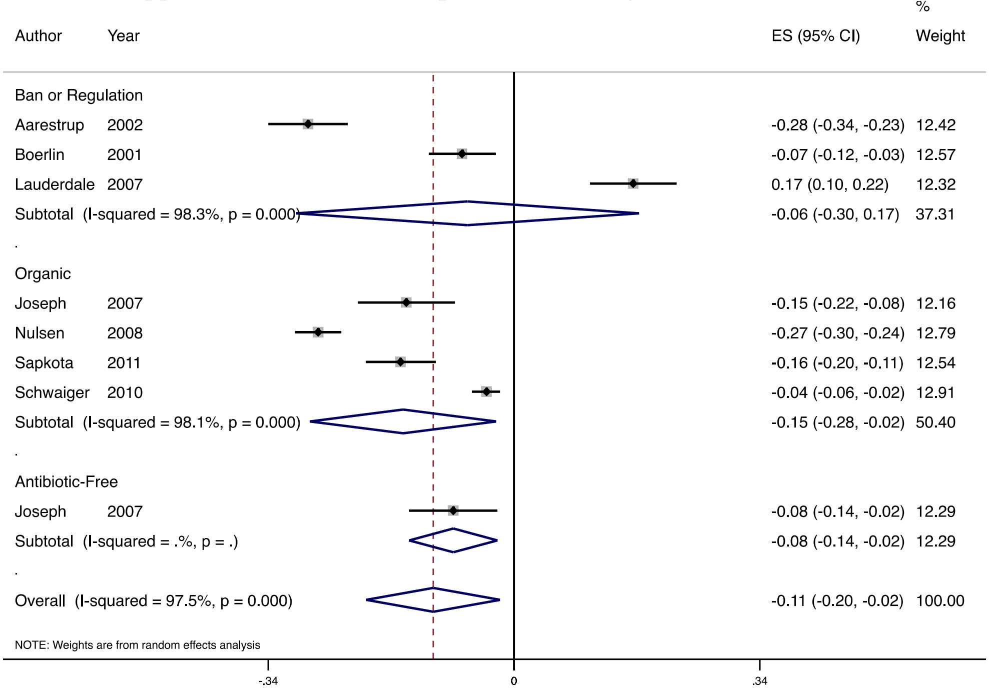


Figure 44: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

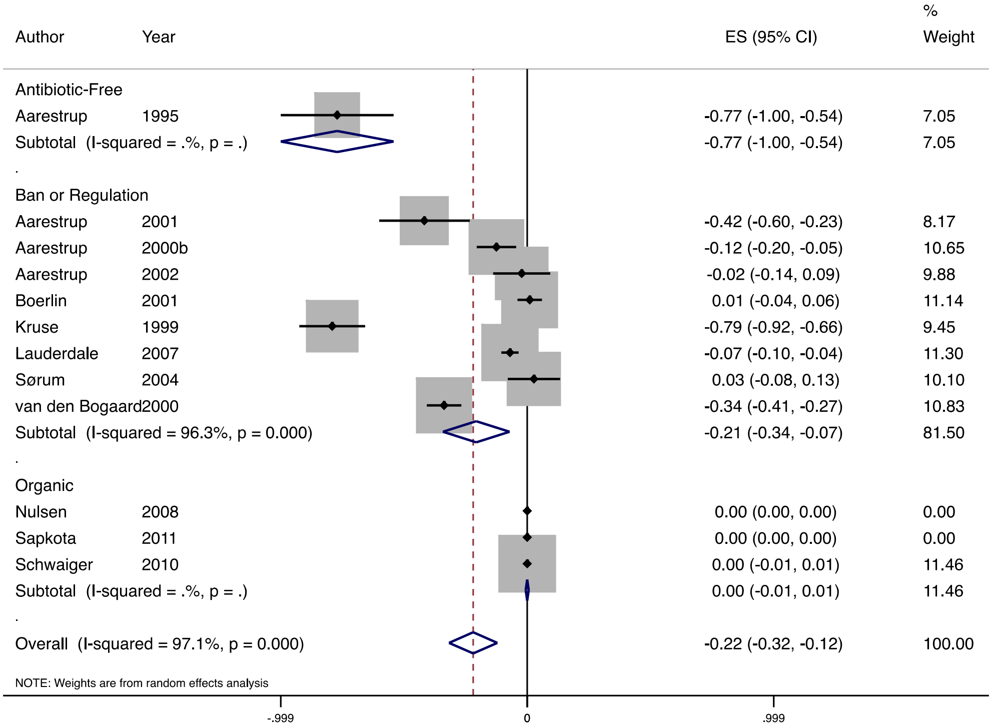


Figure 45: Forest plot of absolute risk differences of antibiotic resistance to glycopeptides for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

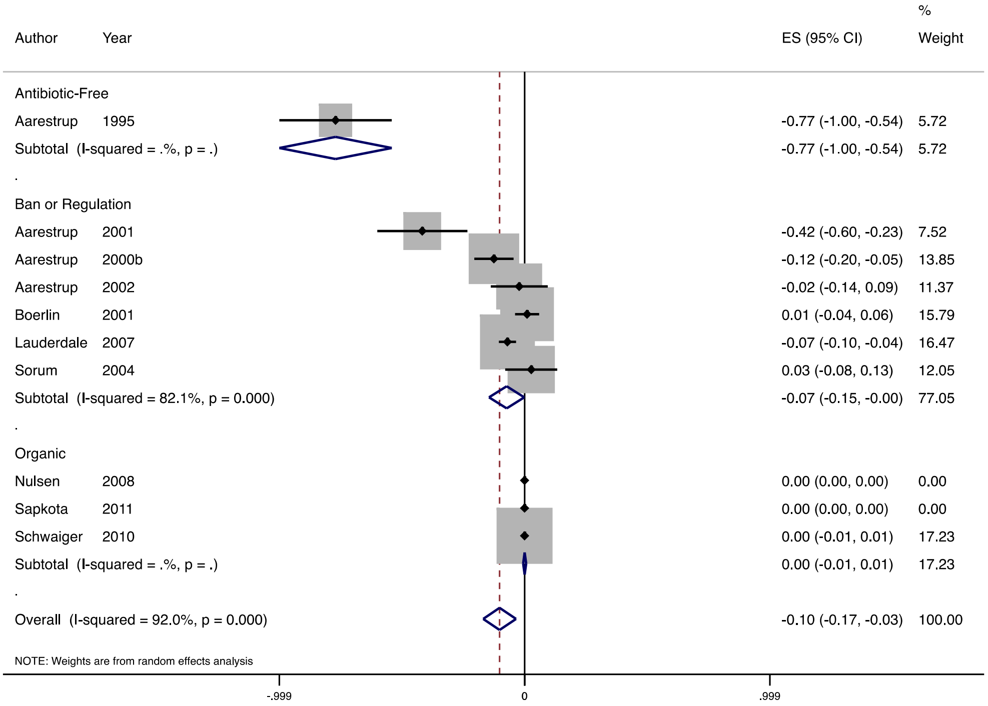


Figure 46: Forest plot of absolute risk differences of antibiotic resistance to macrolides for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

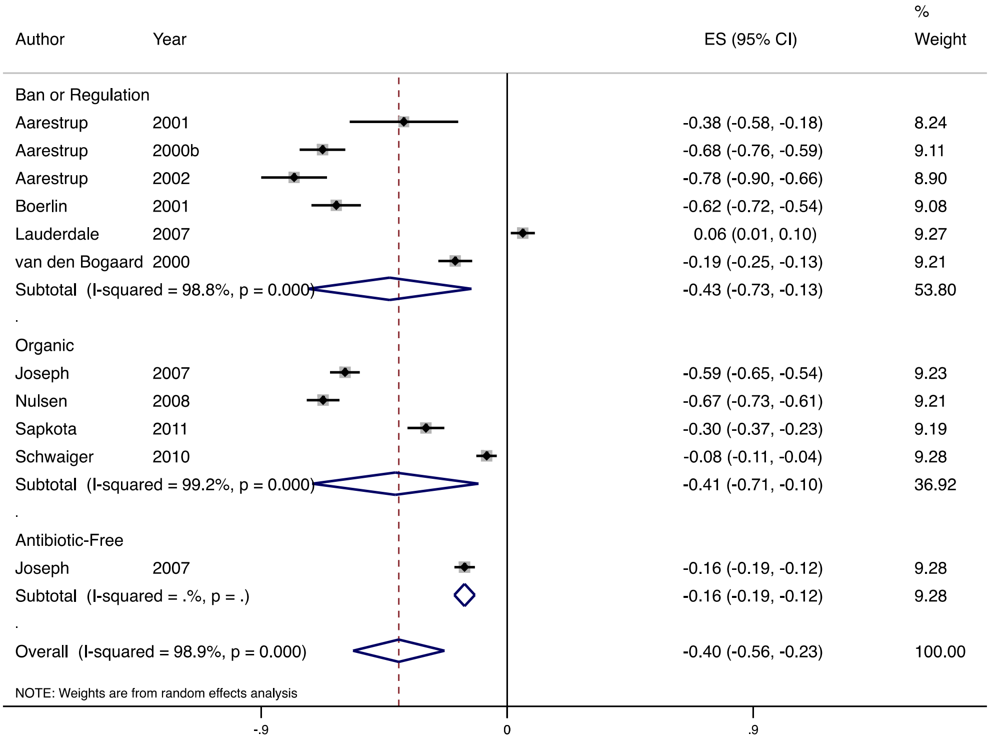


Figure 47: Forest plot of absolute risk differences of antibiotic resistance to penicillins for *Enterococcus* spp isolates in faecal samples, stratified by intervention

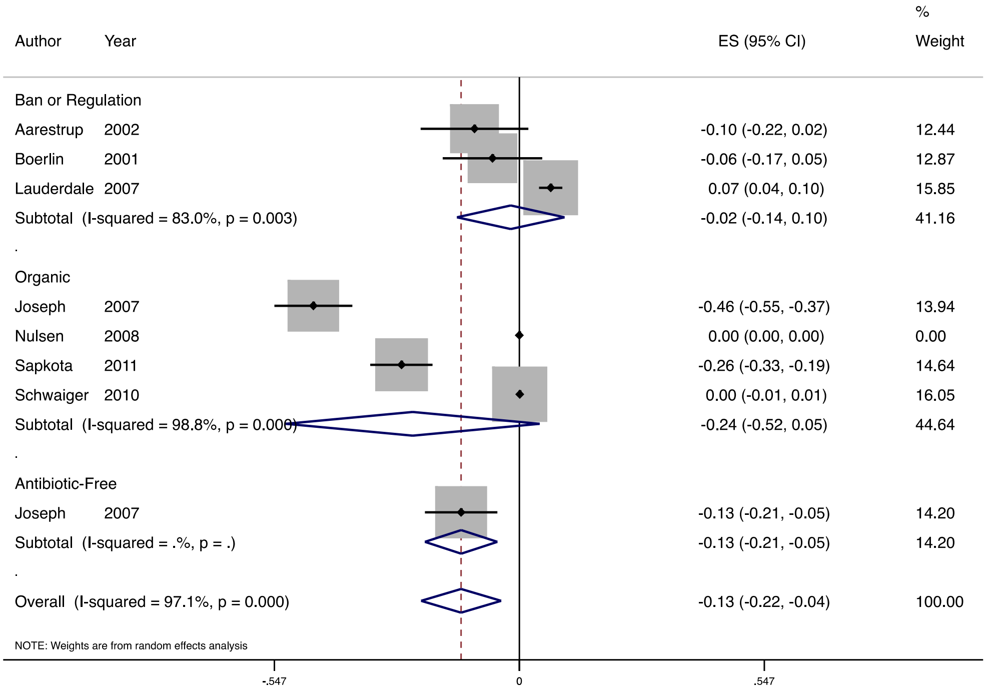


Figure 48: Forest plot of absolute risk differences of antibiotic resistance to streptogramins for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

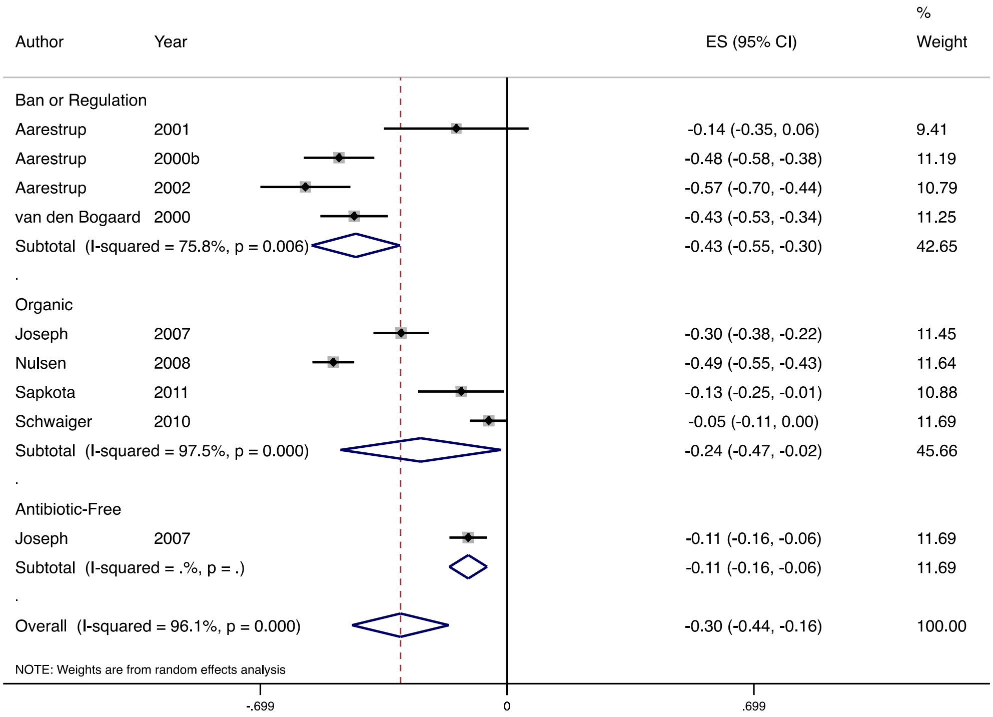


Figure 49: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for *Enterococcus* spp. isolates in faecal samples, stratified by intervention

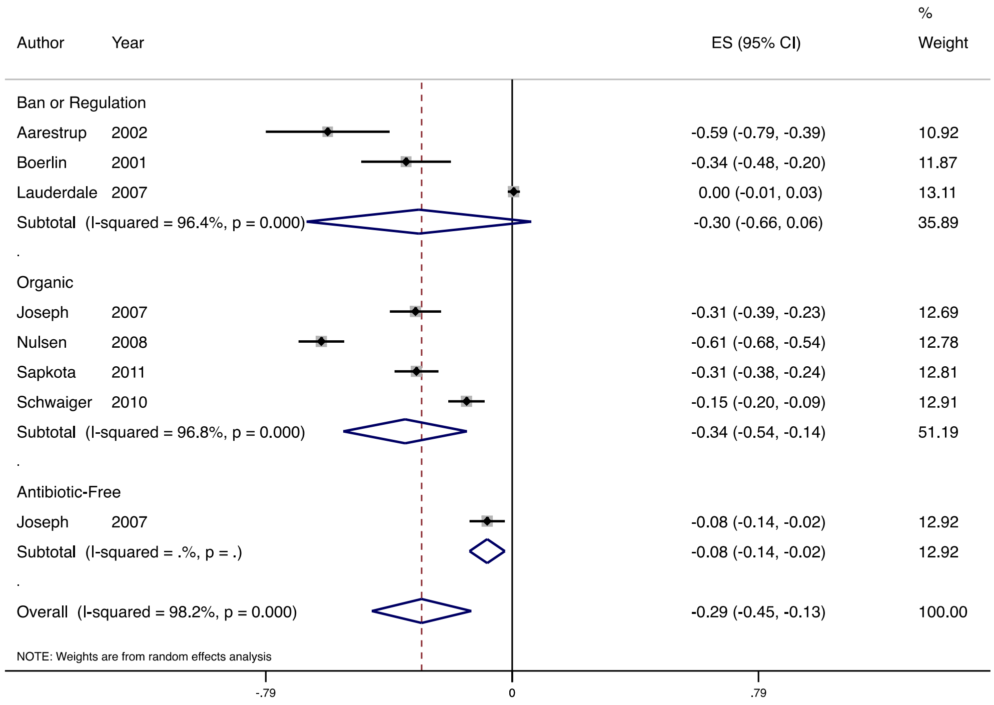


Figure 50: Forest plot of absolute risk differences of antibiotic resistance to aminoglycosides for *Campylobacter* spp. isolates in faecal samples, stratified by intervention

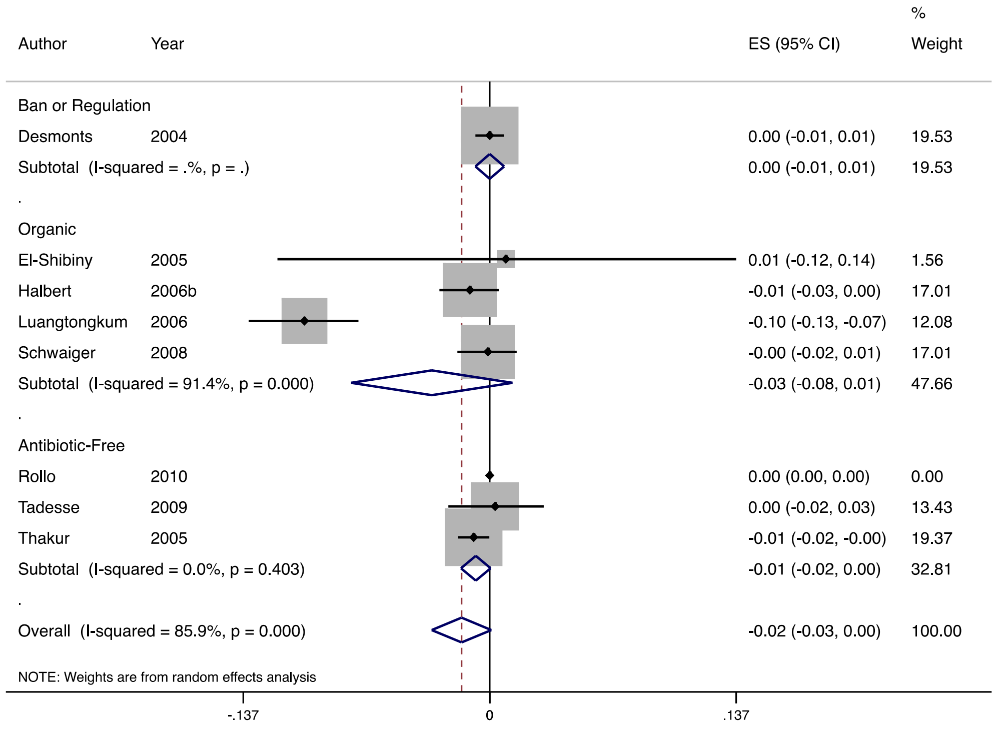


Figure 51: Forest plot of absolute risk differences of antibiotic resistance to amphenicols for *Campylobacter* spp. isolates in faecal samples, stratified by intervention

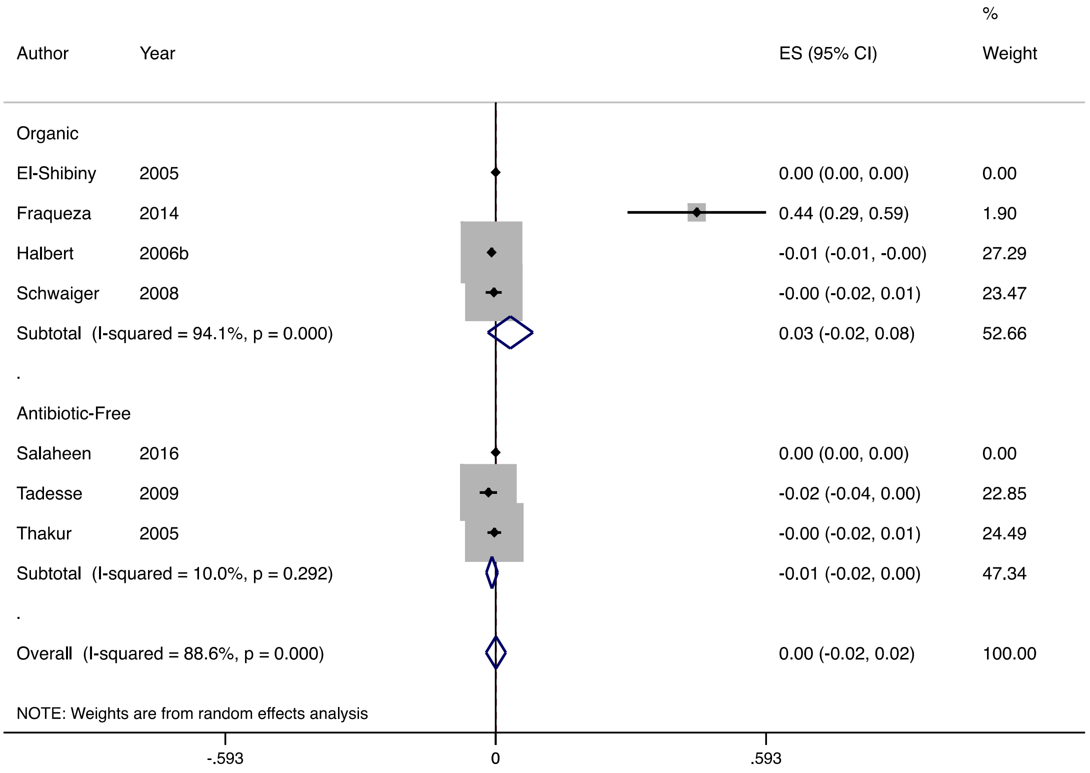


Figure 52: Forest plot of absolute risk differences of antibiotic resistance to macrolides for *Campylobacter* spp. isolates in faecal samples, stratified by intervention

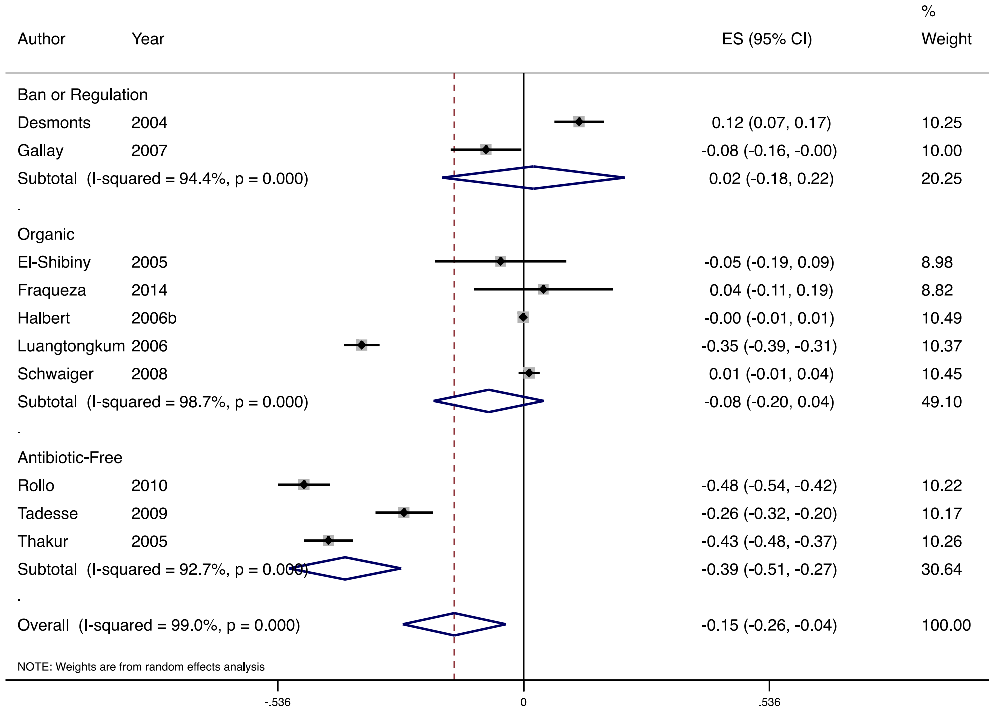


Figure 53: Forest plot of absolute risk differences of antibiotic resistance to penicillins for *Campylobacter* spp. isolates in faecal samples, stratified by intervention

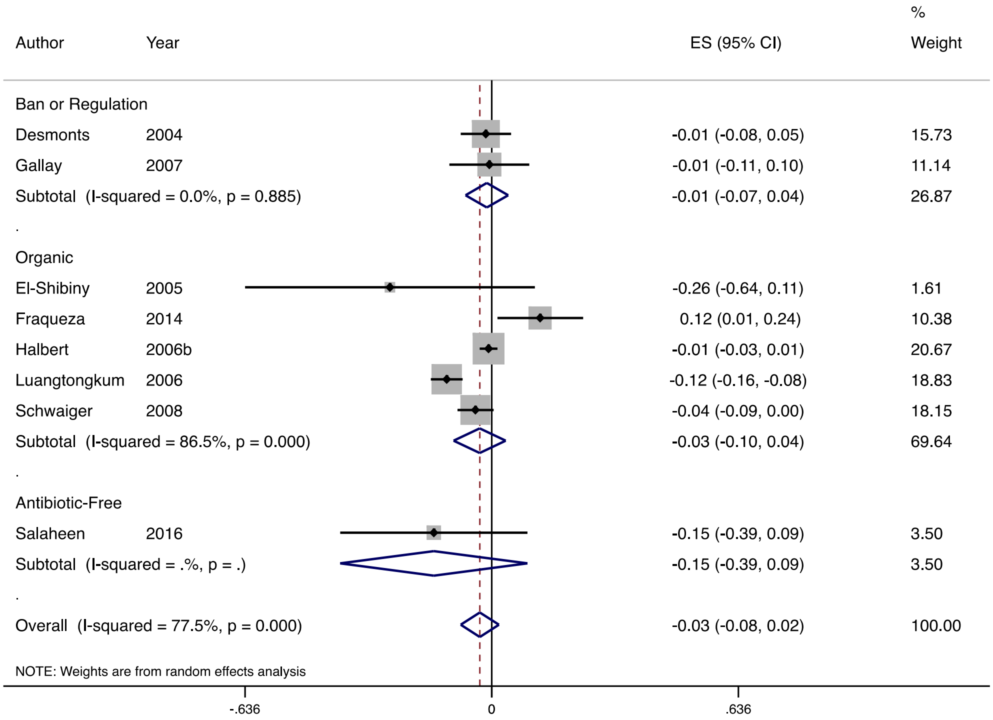


Figure 54: Forest plot of absolute risk differences of antibiotic resistance to quinolones for *Campylobacter* spp. isolates in faecal samples, stratified by intervention

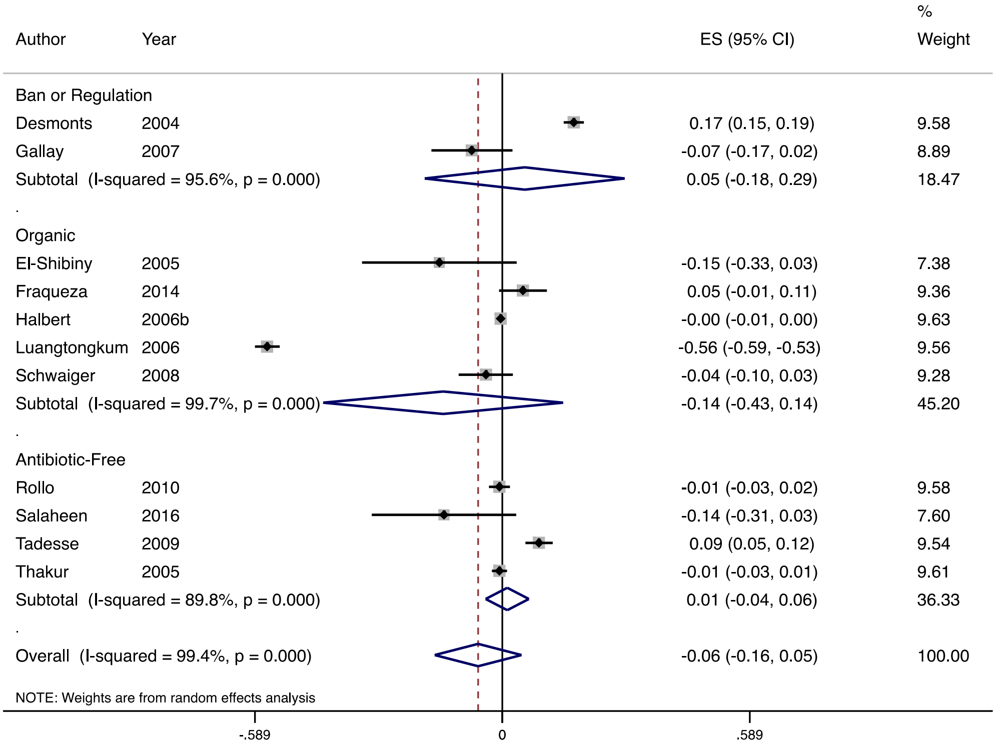
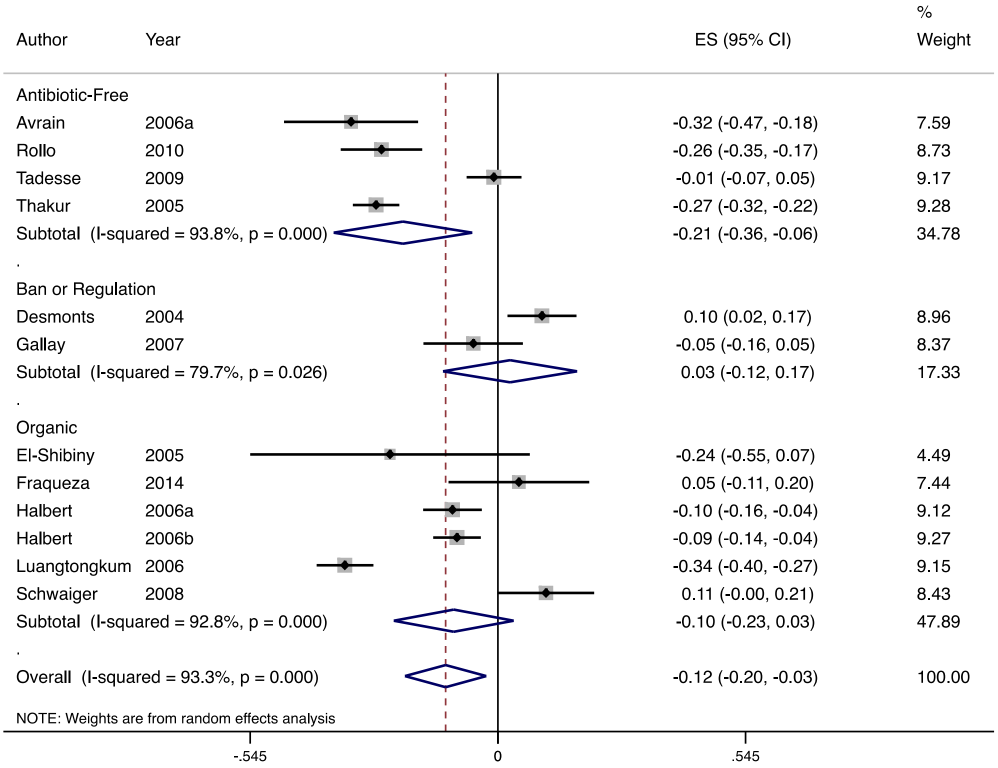


Figure 55: Forest plot of absolute risk differences of antibiotic resistance to tetracyclines for *Campylobacter* spp. isolates in faecal samples, stratified by intervention



1.3 Supplemental report to the “Restriction in the use of antibiotics in food animals and antibiotic resistance in food animals and humans” (University of Calgary, Canada)

Authors	Karen L. Tang, Niamh P. Caffrey, Diego B. Nóbrega, Susan C. Cork, Paul E. Ronksley, Herman W. Barkema, Alicia J. Polachek, Heather Ganshorn, Nishan Sharma, James D. Kellner, William A. Ghali
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Submission	March 2017

The full report to the World Health Organization (WHO), titled “Restriction in the use of antibiotics in food animals and antibiotic resistance in food animals and humans—a systematic review and meta-analysis” was completed in October 2016. Findings from this completed review are important in informing the development of WHO guidelines on the use and restriction of antibiotics in food animals. For further refinement in the creation of these guidelines and recommendations, the WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) has requested the following supplemental work:

1. Update of the literature search, to identify studies published since the search strategy was last run in July 2016
2. Stratified analysis of the pooled reduction in antibiotic resistance, by the type of antibiotic use that is restricted or targeted by interventions
3. Data extraction of the studies included in the systematic review for unintended consequences or harms from interventions that restrict antibiotic use

This report presents the results of the requested supplemental work.

I. Update of Literature Search

The search strategy described in the original systematic review was re-run in January 2017 in the following electronic databases, to capture studies published since our July 2016 search:

- Agricola – Ebsco Platform
- AGRIS (http://agris.fao.org)
- BIOSIS Previews – Web of Knowledge Platform
- CAB Abstracts – Ebsco Platform
- MEDLINE – Ovid Platform (Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R)
- EMBASE – Ovid Platform
- Global Index Medicus (http://www.globalhealthlibrary.net/): The non-MEDLINE indices included: AIM (AFRO), LILACS (AMRO/PAHO), IMEMR (EMRO), IMSEAR (SEARO), WPRIM (WPRO), WHOLIS (KMS), and SciELO.
- ProQuest Dissertations – ProQuest Platform
- Science Citation Index – Web of Knowledge Platform

A total of 191 citations were identified. Two authors (KT and NC) reviewed all abstracts for potential eligibility for inclusion into the systematic review. Any abstract that (a) reported on original research, (b) described an active intervention that aimed to limit antibiotic use in animals, and (c) described antibiotic resistance in animals or humans were selected for full-text review. Fifteen studies were selected for full-text review, of which four met the pre-specified criteria, as described in the original report, for inclusion into the systematic review.[1-4] All four were animal studies, only one of which could be included into the main set of meta-analyses.[3]

We have updated the systematic review and meta-analysis to include these four studies and a revised full report, dated March 7, 2017, has been produced and provided to the WHO. The findings and conclusions in the updated report are unchanged from those in the original report.

II. Stratified Analysis by Intervention Type

To conduct stratified analysis, interventions needed to be classified based on the type of antibiotic use that was targeted. The following classification scheme was thereby created, with input and feedback from WHO AGISAR:

1 – Restriction on the use of all antibiotics

2a – Antibiotic class-specific restriction, or restriction on the use of one or more (but not all) classes of antibiotics, for all indications of use

2b – Antibiotic-specific restriction, or restriction on the use of one or more individual antibiotics, for all indications of use

3 – Restriction on the use of antibiotics for all non-therapeutic indications including growth promotion, prophylaxis, and metaphylaxis *(Treatment of diseased animals permitted only)*

4 – Restriction on the use of antibiotics for the non-therapeutic indications of growth promotion and prophylaxis *(Treatment and metaphylaxis permitted)*

5 – Restriction on the use of antibiotics for purposes of growth promotion only *(Treatment, metaphylaxis, and prophylaxis permitted)*

6 – Undetermined: Inability to classify the intervention type into one of the above categories, or where the indication for antibiotic use that is targeted by the intervention is not specified

Of particular note, every study included into the systematic review assessed an intervention that restricted the use of antibiotics. Studies that did not specify the type of antibiotic use or indication that was targeted in this restriction were classified as “Undetermined” (Category 6). This included studies, for example, that compared regions or farms using “more” versus “less” antibiotics with no indication that was specifically targeted or described, or studies that assessed the impact of reducing antibiotic use in a jurisdiction without delineating how this was achieved.

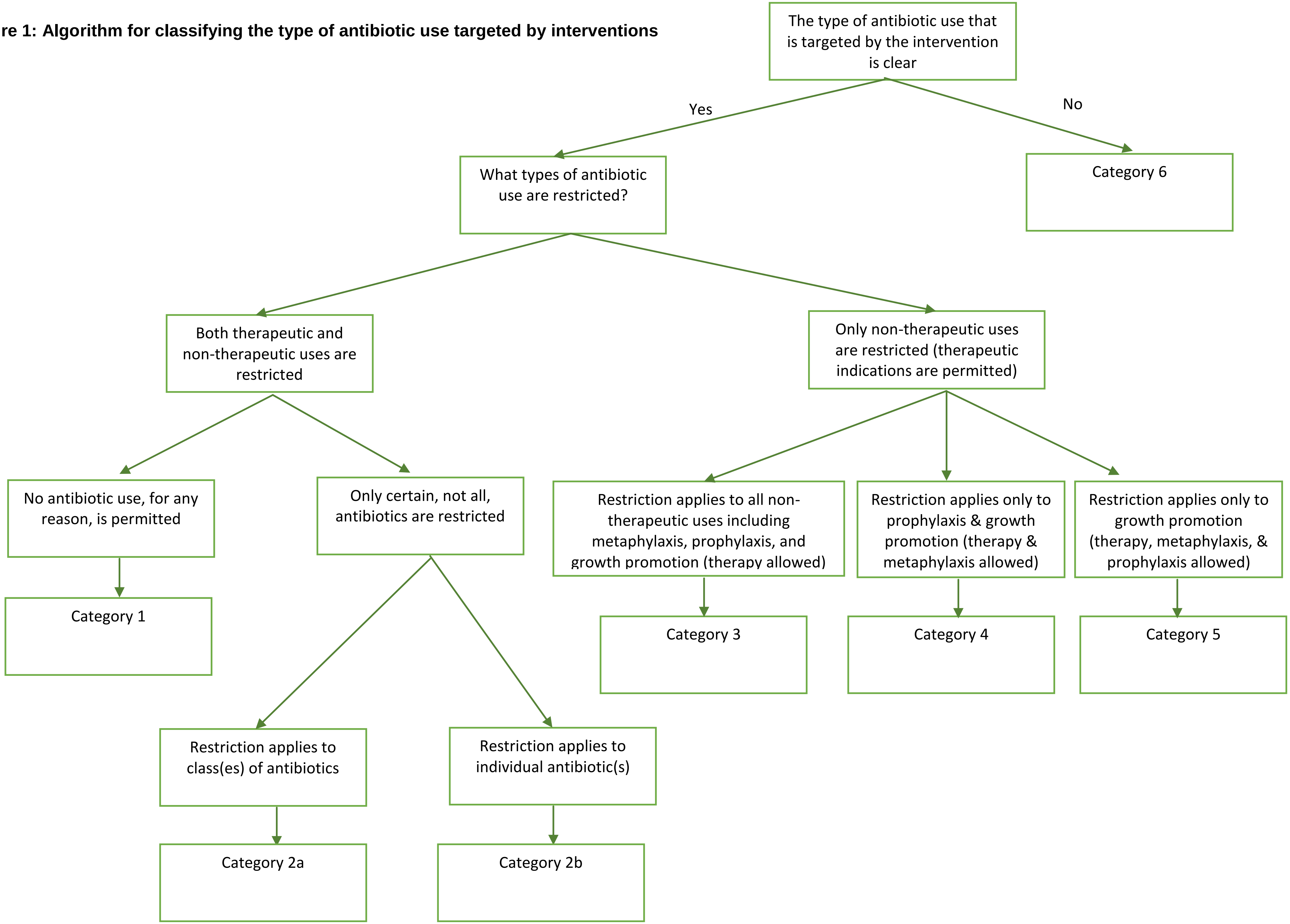
Each category in the classification scheme is mutually exclusive. If a single study included more than one intervention, then each intervention was classified separately based on the above approach. Table 1 outlines the definitions used by the classification scheme.

Table 1: Definitions for terms used in the classification scheme for interventions

Terminology	**Definition**
Antibiotic growth promoter	Administration of sub-therapeutic doses of antibiotics to stimulate growth in animals or to increase feed efficiency.[5,6]
Non-therapeutic antibiotic use	Administration of antibiotics to animals without identifiable infectious disease.[5] This includes antibiotic use for growth promotion, disease prophylaxis, and metaphylaxis.
Metaphylaxis	Treatment of a group of animals without evidence of disease, which are in close contact with other animals that do have evidence of infectious disease.[7]
Prophylaxis	Administration of antibiotics to animals at high risk of infectious disease (but without current disease and where there is no known disease in the herd or flock).[5] Prophylaxis is commonly used when environmental conditions or changes portend increased risk for infection. Examples of such conditions include transport of animals and confining animals to small, crowded spaces.[5]
Therapeutic antibiotic use	Administration of antibiotics to treat animals with clinical evidence of infectious disease only.[5,6]

Figure 1 illustrates the corresponding decision rules that were followed, to apply the above classification scheme to each study.

Figure 1: Algorithm for classifying the type of antibiotic use targeted by interventions


The type of antibiotic use that is targeted by the intervention is clear
Yes
No
What types of antibiotic use are restricted?
Category 6
Both therapeutic and non-therapeutic uses are restricted
Only non-therapeutic uses are restricted (therapeutic indications are permitted)
No antibiotic use, for any reason, is permitted
Only certain, not all, antibiotics are restricted
Restriction applies to all non-therapeutic uses including metaphylaxis, prophylaxis, and growth promotion (therapy allowed)
Restriction applies only to prophylaxis & growth promotion (therapy & metaphylaxis allowed)
Restriction applies only to growth promotion (therapy, metaphylaxis, & prophylaxis allowed)
Category 1
Category 3
Category 4
Category 5
Restriction applies to class(es) of antibiotics
Restriction applies to individual antibiotic(s)
Category 2a
Category 2b

A. Results – Classification of study interventions

Table 2 presents the categorization of interventions by type of antibiotic use being targeted for restriction.

Table 2: Type of antibiotic use targeted by interventions in 179 animal studies

Type of antibiotic use limited by intervention	Number of studies
Restriction on the use of all antibiotics	69
Restriction on the use of one or more (but not all) classes of antibiotics, for all indications of use	6
Restriction on the use of one or more individual antibiotics, for all indications of use	6
Restriction on the use of antibiotics for all non-therapeutic indications including growth promotion, prophylaxis, and metaphylaxis *(Treatment of diseased animals permitted only)*	36
Restriction on the use of antibiotics for the non-therapeutic indications of growth promotion and prophylaxis *(Treatment and metaphylaxis permitted)*	1
Restriction on the use of antibiotics for the non-therapeutic indication of growth promotion *(Treatment, metaphylaxis, and prophylaxis permitted)*	27
Undetermined: Inability to classify the intervention type into one of the above categories, or where the indication for antibiotic use that is targeted by the intervention is not specified	39

*Note: 5 studies included two different interventions

Of the 179 animal studies included in the systematic review, 69 restricted all uses of antibiotics, 36 studies restricted use of antibiotics for all non-therapeutic purposes, while 27 restricted the use of antibiotics for growth promotion only. A total of 39 studies could not be classified based on the type of antibiotic use targeted by the intervention. An index of the 179 animal studies, their corresponding references from the original report, and their assigned classifications of interventions is presented in Supplemental Table 1 in the Appendix to this supplemental report.

Table 3 presents the categorization of interventions by type of antibiotic use being targeted for restriction, for human studies.

Table 3: Type of antibiotic use targeted by interventions in 21 human studies

Type of antibiotic use limited by intervention	Number of studies
Restriction on the use of all antibiotics	5
Restriction on the use of one or more (but not all) classes of antibiotics, for all indications of use	1
Restriction on the use of one or more individual antibiotics, for all indications of use	1
Restriction on the use of antibiotics for all non-therapeutic indications including growth promotion, prophylaxis, and metaphylaxis *(Treatment of diseased animals permitted only)*	2
Restriction on the use of antibiotics for the non-therapeutic indications of growth promotion and prophylaxis *(Treatment and metaphylaxis permitted)*	0
Restriction on the use of antibiotics for the non-therapeutic indication of growth promotion *(Treatment, metaphylaxis, and prophylaxis permitted)*	7
Undetermined: Inability to classify the intervention type into one of the above categories, or where the indication for antibiotic use that is targeted by the intervention is not specified	5

Of the 21 human studies, five restricted all uses of antibiotics, two restricted antibiotic use for all non-therapeutic indications, and seven restricted use of antibiotics for growth promotion only. Five studies could not be classified based on the type of antibiotic use targeted by the intervention. An index of the 21 human studies, their corresponding references from the original report, and their assigned classifications of interventions is presented in Supplemental Table 2 in the Appendix to this supplemental report.

B. Results – Stratified analysis

Similar to the stratified analysis conducted in the original systematic review and meta-analysis, stratified meta-analysis was performed for all studies amenable to meta-analysis, ignoring specific bacterial species, sample types, units of analysis, and antibiotic classes. Supplemental Table 1 in the Appendix to this supplemental report lists the individual animal studies amenable to stratified meta-analysis. Table 4 outlines the results from meta-analysis stratified by the type of antibiotic use targeted by interventions in animal studies.

Table 4: Stratified meta-analysis for animal studies, by intervention type

Variable by which stratification conducted	Number of studies	Pooled absolute risk difference (95% CI)
Restriction on the use of all antibiotics	39	-0.18 (-0.22, -0.14)
Restriction on the use of one or more (but not all) classes of antibiotics, for all indications of use	4	0.04 (-0.12, 0.20)
Restriction on the use of one or more individual antibiotics, for all indications of use	5	-0.02 (-0.24, 0.19)
Restriction on the use of antibiotics for all non-therapeutic indications including growth promotion, prophylaxis, and metaphylaxis *(Treatment of diseased animals permitted only)*	26	-0.08 (-0.11, -0.06)
Restriction on the use of antibiotics for the non-therapeutic indications of growth promotion *(Treatment, metaphylaxis, and prophylaxis permitted)*	15	-0.29 (-0.40, -0.19)
Undetermined: Inability to classify the intervention type into one of the above categories, or where the indication for antibiotic use that is targeted by the intervention is not specified	17	-0.13 (-0.18, -0.08)

*Meta-regression p-value for the 6 categories, p=0.35

Stratified meta-analysis must be interpreted with some caution, due to the lower numbers of studies that can be included and the overlapping confidence intervals in the pooled estimates across strata. With these caveats in mind, we would propose three high-level observations from the stratified analysis, which we summarize below, followed by further elaboration:

1. The type of antibiotic use targeted by interventions is not specified in many of the studies identified by our search. This finding underlines the need for better characterization of interventions in future research, and perhaps even more importantly, in the development of future policy and regulations.
2. There is some suggestion that the interventions that target only specific antibiotic classes or specific antibiotic drugs may have less effect on antibiotic resistance than do antibiotic restrictions covering all classes.
3. Among antibiotic restriction interventions that target all classes, there does not seem to be any advantage of complete bans preventing *any use* relative to restrictions that still permit therapeutic and prophylactic use.

In 39 of 179 animal studies and 5 of 21 human studies, the type of antibiotic use that was targeted by interventions could not be determined. For the majority of these studies, antibiotic resistance was compared between groups having “higher” versus “lower” antibiotic use, without further delineation as to how antibiotics were being used or restricted in both groups. Though our stratified analysis suggests that lower overall antibiotic use does seem to be associated with less antibiotic resistance in this “Undetermined” group, the development of policies and regulations on antibiotic use requires more specific information on interventions, to explore whether certain interventions appear to be more or less effective than others. Therefore, future research should focus on better characterizing interventions that restrict antibiotic use.

Secondly, our stratified analysis results suggest that broad interventions that globally restrict the use of all classes of antibiotics may be more effective in reducing antibiotic resistance, compared to interventions that narrowly restrict the use of a few specific antibiotics or antibiotic classes. For example, the absolute risk differences for interventions that broadly restrict antibiotics across different classes range from -0.08 to -0.29. That is, the proportion of bacteria with antibiotic resistance is 8 to 29% lower in intervention versus comparator groups, with such broad interventions. In contrast, the absolute risk differences for interventions that restrict only a single or a few antibiotic(s) or antibiotic class(es) range between -0.02 and 0.04, with confidence intervals overlapping 0, indicating that there is no difference in antibiotic resistance in intervention versus comparator groups. Interventions that restrict specific antibiotics and antibiotic classes may therefore be less beneficial than global restrictions that are not confined to specific antibiotics and antibiotic classes.

Lastly, our results suggest that full restriction of antibiotics (where antibiotics cannot be used for any indication, including non-therapeutic and therapeutic purposes) does not appear to be superior to interventions that do allow for therapeutic use of antibiotics as well as for metaphylaxis and prophylaxis of animals. The absolute risk difference of antibiotic resistance with full antibiotic restriction was -0.18, compared to absolute risk differences between -0.08 and -0.29 for partial restrictions only. Restricted antibiotic use that permits the treatment of diseased animals and/or diseased herds does not seem to undermine efforts to reduce antibiotic resistance.

It is difficult to formulate more precise conclusions beyond the above three observations. For example, the reduction in antibiotic resistance appears to be stronger for interventions that restrict growth promotion only (RD -0.29 [95% CI -0.40, -0.19]), compared with interventions that are even more restrictive, such as those disallowing prophylaxis or metaphylaxis in addition to disallowing growth promotion (RD -0.08 [95% CI -0.11, -0.06]), or disallowing all uses of antibiotics including for therapy (RD -0.18 [95% CI -0.22, -0.14]). However, these differences in the absolute risk reductions of antibiotic resistance across interventions may be artefactual. The pooled prevalence of antibiotic resistance in comparator groups for interventions restricting use of growth promoters is 0.48 (95% 0.23, 0.73), compared to 0.13 (95% CI 0.11, 0.16) in the comparator groups for interventions that restrict all non-therapeutic uses of antibiotics. That is, the pooled “baseline” prevalence in the comparator groups is not the same across all intervention types. There is greater potential for reduction of antibiotic resistance when there is a higher baseline prevalence of resistance, such as in the case for interventions restricting the use of growth promoters. Therefore, differences in pooled effect estimates across different interventions that broadly restrict antibiotics may spurious; we cannot comment on a specific intervention being superior to others, so long as restrictions are not confined to a single class of antibiotics.

Corresponding stratified meta-analysis based on intervention type was also conducted for human studies (Table 5). Supplemental Table 2 (to be requested upon request) in the Appendix to this supplemental report lists the individual human studies amenable to stratified meta-analysis.

Table 5: Stratified meta-analysis for human studies, by intervention type

Variable by which stratification conducted	Number of studies	Pooled absolute risk difference (95% CI)
Restriction on the use of all antibiotics	3	-0.43 (-1.25, 0.40)
Restriction on the use of antibiotics for all non-therapeutic indications including growth promotion, prophylaxis, and metaphylaxis *(Treatment of diseased animals permitted only)*	1	-0.08 (-0.20, 0.04)
Restriction on the use of antibiotics for the non-therapeutic indications of growth promotion *(Treatment, metaphylaxis, and prophylaxis permitted)*	6	-0.13 (-0.20, -0.06)
Undetermined: Inability to classify the intervention type into one of the above categories, or where the indication for antibiotic use that is targeted by the intervention is not specified	3	-0.31 (-0.56, -0.05)

*Meta-regression p-value for the 4 categories, p=0.55

Due to the relatively small numbers of human studies and the wide and overlapping confidence intervals in subcategories, we caution against attempting to draw specific inferences regarding relative effects of the different intervention types in human studies.

III. Information on Potential Unintended Consequences of Interventions That Restrict Antibiotic Use

Data were extracted from the studies included in the systematic review, regarding potential harms stemming from interventions that restrict antibiotic use. Categories of potential harms included: 1) increased use of antibiotics (such as increased need for antibiotics for treatment purposes), 2) adverse effects on human health, 3) decrease in food and protein availability, 4) food safety, 5) adverse effects on animal health and welfare, 6) adverse effects on animal production, and 7) economic consequences.

Only 48 studies in total (all animal studies, two of which also examined antibiotic resistance in the human population) reported any data on the presence or absence of potential harms of interventions that restrict antibiotic use. Of these, 32 explicitly had at least one of the aforementioned potential harms as a primary research objective. One study examined animal production consequences as a secondary objective.[8] The other 15 studies reported potential harms in the discussion section without pre-specifying these as objectives. No studies reported adverse effects on human health or on food and protein availability. Table 6 presents a summary of the extent to which information on harms is reported in the identified studies. Of note, a single study could report on more than one potential harm.

Table 6: Potential harms reported by animal and human studies

Potential harms	Number of animal studies (N=179)	Number of human studies (N=21)
Increased use of antimicrobials	5	2
Adverse effects on human health	0	0
Decrease in food or protein availability for human consumption	0	0
Food safety	34	0
Adverse effects on animal health	5	1
Animal production	4	1
Economic (cost of animal production or national economy)	3	1
No data reported on potential harms	131	19

Table 7 summarizes the specific unintended consequences reported by individual studies.

Table 7: Specific reporting in identified studies relating to potential harms from interventions restriction antibiotic use

	Antibiotic use	Food safety	Adverse effects on animal health	Animal production	Economic
Aarestrup 2001	↑ Other AGPs				
Abdalrahman 2015		<-> *Staphylococcus aureus*			
Alali 2010		↓ *Salmonella* spp.			
Álvarez Fernández 2012		<-> Psychrotrophs <-> Enterobacteriaceae <-> *Pseudomonas* spp. <-> *Enterococcus* spp. <-> Molds and yeasts ↓ *Staphylococcus* spp.			
Álvarez Fernández 2013		<-> Psychrotrophs <-> Coliforms			
Berge 2009			↓ Diarrhea ↑ Respiratory disease	↑ Weight gain	↓ Treatment costs
Bombyk 2008		↑ *Staphylococcus aureus*			
Coalition for Animal Health	↑ Antibiotic therapeutic use ↓ Total use of antibiotics				
Cui 2004		↑ *Salmonella* spp.			
Cui 2005		↑ *Salmonella* spp. ↑ *Campylobacter* spp.			
Dorado-Garcia 2015b	↓ Group treatment ↑ Individual treatment ↓ Total use of antibiotics		<-> Mortality <-> Mean mortality age	<-> Carcass weight ↑ Production cycle duration	↑ Vet costs

	Antibiotic use	Food safety	Adverse effects on animal health	Animal production	Economic
El-Shibiny 2005		↑ *Campylobacter coli*			
Garmo 2010				↑ Parity ↓ Milk yield	
Ge 2004		<-> *Campylobacter* spp.			
Gebreyes 2006		↑ *Salmonella* spp.			
Han 2009		<-> *Campylobacter* spp.			
Heuer 2001		↑ *Campylobacter* spp.			
Jensen 2014	↑ Therapeutic antibiotic use				
Keelara 2013		↑ *Salmonella* spp.			
Lee 2013		↑ *Salmonella* spp.			
Lestari 2009		<-> *Salmonella spp.*			
Luangtongkum 2006		<-> *Campylobacter* spp.			
Mazengia 2014		<-> *Salmonella spp.*			
Miranda 2007		↑ *Enterococcus* spp.			
Miranda 2008		↑ Enterobacteriaceae			
Miranda 2008b		↑ *Escherichia coli*			
Miranda 2008c		↑ *Escherichia coli* <-> *Staphylococcus aureus* <-> *Listeria monocytogenes*			
Miranda 2009		<-> *Escherichia coli* <-> *Salmonella* spp. <-> *Staphylococcus aureus* <-> *Listeria monocytogenes*			
Miranda 2009b		<-> Microbiological acceptability			
Mollenkopf 2014		<-> *Salmonella* spp. <-> *Campylobacter* spp.			

	Antibiotic use	Food safety	Adverse effects on animal health	Animal production	Economic
Morley 2011	↑ Median amount of antibiotics used per pen ↓ Total use of antibiotics			↑ Feeding time to achieve same weight*	↑ Energy and resources for production of feed* ↑ Land space for urine and fecal output*
Noormohamed 2014		↓ *Campylobacter* spp.			
Park 2012			↑ Intramammary infections at parturition <-> Intramammary infections at dry-off		
Peng 2016		↑ *Salmonella* spp.			
Pol 2007		↑ Bacterial contaminants	↑ Intramammary infections		
Price 2005		<-> *Campylobacter* spp.			
Price 2007		<-> *Campylobacter* spp.			
Roesch 2006			<-> Mastitis		
Salaheen 2016		↑ *Campylobacter* spp.			
Schwaiger 2008					
Schwaiger 2010					
Siemon 2007					
Tadesse 2009					
Tamang 2015					
Teramoto 2016		<-> *Staphylococcus. aureus*			
Thakur 2005		<-> *Campylobacter* spp.			
Zhang 2010		<-> *Escherichia coli* <-> *Enterococcus* spp.			

	Antibiotic use	Food safety	Adverse effects on animal health	Animal production	Economic
Zhang 2011		↑ *Salmonella* spp. ↓ Coliforms <-> *Escherichia coli* <-> *Enterococcus* spp.			

Where Red = favors comparator group; Green = favors intervention group; Yellow = no difference between intervention and comparator groups

Abbreviations: AGP – Antibiotic growth promoters; ↑= increased in the intervention compared to the comparator group; ↓= decreased in the intervention compared to the comparator group; and <-> = no difference between the intervention and comparator groups

* Combined effect of a bundled intervention that restricted the use of antibiotics, hormone implants, and anti-helmintics

A. Antibiotic use

Five studies reported on potential unintended consequences with regard to the total amount of antibiotics used. One study reported that when one antibiotic growth promoter was banned, there tended to be an increased use of other permitted antibiotic growth promoters until the use of these, too, was restricted.[9] The other four studies reported that when antibiotic use was restricted, this resulted in increased administration of antibiotics to individual animals for therapeutic purposes, but that the total amount or volume of antibiotics used nevertheless decreased.[8,10-12]

B. Food safety

The most widely reported potential unintended consequence was in the domain of food safety, with 34 studies reporting on this outcome. Of these, 14 (41%) found that interventions that restricted antibiotic use resulted in increased contamination with bacteria (including *Salmonella* spp., *Campylobacter* spp., and Enterobacteriaceae) in the retail meats produced. Fifteen of 34 studies (45%) reported no difference in contamination rates between food products from intervention and comparator groups. A smaller percentage of studies (12%) demonstrated either variable results within studies or a lower level of contamination of meats in intervention versus comparator groups. The clinical and public health significance of these findings are unclear, especially as to what extent adequate preparation and cooking can mitigate the risk of bacterial contamination of raw retail meat, and whether higher bacterial contamination translates into increased clinical and zoonotic disease.

C. Animal health

Only five studies reported potential adverse effects on animal health.[8,13-16] Three such studies were specific to dairy herds, showing variable results. Two of the three reported higher prevalence of intra-mammary infections when the use of antibiotics is restricted (though one study indicated that the higher prevalence was significant only at parturition but not the dry-off period),[14,15] while the third study showed no difference in the prevalence of mastitis between intervention and comparator groups.[16] Berge et al. reported an increase in respiratory disease but decrease of diarrhea in calves where antibiotics used for prophylaxis and growth promoters were restricted.[13] Lastly, Dorado-Garcia et al. reported no difference in mortality or mean mortality age in intervention versus comparator groups.[8]

D. Animal production

Studies reporting on the effects of antibiotic restriction on animal production again demonstrated variable results. One study indicated that such interventions resulted in greater weight gain (from reduced diarrhea) in intervention groups,[13] while two studies indicated that animal production was adversely affected by antibiotic restriction, with increased feeding time (to achieve a target weight) or increased production cycle duration in intervention groups.[8,12] There may also be effects on parity and milk yield, with antibiotic restriction being associated with increased parity but lower milk yield in one study.[17]

E. Costs and economics

Only three studies reported potential economic consequences of antibiotic restriction interventions. One study showed that restriction in antibiotic use, in combination with restrictions in the uses of hormone implants and anti-helmintics, may increase feeding time to reach target weight in animals, leading to increases in the need for land for disposal of waste, and increases in energy consumption for animal food production.[12] It is difficult to disentangle the extent to which these unintended consequences in animal production and costs are attributable to the antibiotic restrictions themselves, versus the co-interventions that were implemented in this study. Other studies show variable economic implications to treatment and veterinary costs, with one study showing an increase while another showing a decrease in such costs.[8,13]

In summary, proportionately few studies included in the systematic review reported on unintended consequences of interventions that restrict antibiotic use. We also note that unintended consequences of antibiotic restriction were not the focus of any study included in the systematic review. Similarly, our search strategy did not explicitly include harms or unintended consequences, and therefore the studies captured in our systematic review may not be a comprehensive reflection of the literature in this area. Given the importance of potential harms in interventions that restrict antibiotic use, a separate systematic review dedicated to this question may be needed to guide policy recommendations. This report is being provided to the WHO as a supplement to the main report, which has also been re-submitted in an updated form on the same date. Given the supplemental nature of the analysis in this report, findings do need to be reviewed in conjunction with the main report, rather than as a stand-alone report focusing on unintended consequences.

IV. Conclusion

The supplemental analysis that has been requested sheds light on various policy-relevant questions. Specifically, in the bacteria studied, broad restrictions covering all antibiotic classes appear to be more effective in reducing antibiotic resistance compared to narrow restrictions of one antibiotic class or drug. Furthermore, complete restrictions on the use of all antibiotics do not seem to be more effective than interventions that allow for appropriate therapeutic use. Regarding potential unintended consequences, there appears to be a recurring finding of somewhat increased use of therapeutic antibiotic courses in individual animals (though an overall reduction in the volume of antibiotics used) with interventions that restrict antibiotic use, and possible implications for food safety given the possible higher prevalence of bacterial contaminants in these food products. These findings are likely to be important to explore further as future guidelines and recommendations on antibiotic use are developed.

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Supplemental Table 1: Index of animal studies, reference numbers, and intervention types

Study	Reference Number in Original Report	Classification of Intervention(s)	Amenable to Meta-Analysis, Stratified by Intervention Type
Aarestrup (1995)	31	1	Yes
Aarestrup (2000a)	32	5	No
Aarestrup (2000b)	34	5	Yes
Aarestrup (2001)	15	5	Yes
Aarestrup (2002)	33	5	Yes
Abdalrahman (2015)	35	6	No
Agersø (2013)	36	2a	Yes
Agga (2015)	37	1	Yes
Alali (2010)	38	1	Yes
Álvarez-Fernández (2012)	40	3	Yes
Álvarez-Fernández (2013)	39	3	Yes
Avrain (2003)	41	5	Yes
Bager (1999)	42	5	No
Barlow (2008)	43	1	No
Barlow (2009)	44	1	No
Bauer-Garland (2006)	45	2b	Yes
Bengtsson (2006)	46	5	No
Bennedsgaard (2006)	47	3	Yes
Boerlin (2001)	48	5	Yes
Bombyk (2007)	50	6	No
Bombyk (2008)	49	1	Yes
Borgen (2000)	51	5	No
Borgen (2001)	52	5	Yes
Boutet (2005)	53	3	Yes
Boyer (2012)	54	3	No
Bunner (2007)	55	1	Yes
Buntenkoetter (2014)	56	3	No
Butaye (1999)	57	5	No
Cho (2006)	59	1	No
Cho (2007)	60	1	Yes
Cicconi-Hogan (2014)	61	1	Yes
CIPARS (2016)	58	2a	No
Coalition for Animal Health (NR)	62	5	No
Cohen Stuart (2012)	63	3, 6	Yes
Cui (2004)	64	1	Yes
Cui (2005)	65	1	Yes
Cuny (2012)	66	1	Yes
Del Grosso (2000)	67	5	Yes
Desmonts (2004)	68	5, 6	Yes
Docic (2003)	69	4	No
Dolejska (2011)	70	2a	No
Dorado-García (2013)	71	6	No
Dorado-García (2015a)	72	6	No
Dorado-García (2015b)	73	6	No
Dorado-García (2016)	207	1	No
Dutil (2010)	74	2b	Yes
El-Shibiny (2005)	75	3	Yes
Emborg (2002)	76	5	No
Fraqueza (2014)	77	3	Yes
Gallay (2007)	78	2a	Yes
Garcia-Migura (2005)	79	3	No

Study	Reference Number in Original Report	Classification of Intervention(s)	Amenable to Meta-Analysis, Stratified by Intervention Type
Garmo (2010)	80	3	Yes
Ge (2004)	81	1	No
Gebreyes (2006)	82	1	Yes
Gellin (1989)	83	1, 3	Yes
Gerzova (2015)	84	3	No
Guarddon (2014)	85	3	Yes
Halbert (2006a)	86	6	Yes
Halbert (2006b)	87	1	Yes
Hammerum (2007)	88	5	No
Han (2009)	89	1	Yes
Harper (2009)	90	6	No
Harvey (2009)	91	6	No
Hässig (2014)	92	6	Yes
Heuer (2001)	93	3	No
Heuer (2002)	94	5	Yes
Hiki (2015)	95	2b	Yes
Hiroi (2012)	96	1	No
Hoogenboom (2008)	97	3	No
Huijbers (2015)	98	3	Yes
Jensen (2014)	99	1	No
Johnson (2007)	100	1	No
Johnston (2002)	101	1	No
Joseph (2007)	103	6	Yes
Joseph (2008)	102	6	Yes
Kassem (2017)	208	1	Yes
Keelara (2013)	104	1	Yes
Kerouanton (2014)	105	3	Yes
Khachatryan (2006)	106	2b	No
Kieke (2006)	107	1	Yes
Kilonzo-Nthenge (2015)	108	1	No
Kola (2012)	110	6	No
Kruse (1999)	111	5	Yes
Kühn (2005)	112	5	Yes
Lam (2012)	113	1	No
Langlois (1983)	114	1	Yes
Langlois (1986)	115	1	Yes
Larsen (1975)	116	1	Yes
Lauderdale (2007)	117	5	Yes
Lebek (1979)	118	6	No
Lee (2013)	119	6	No
LeJeune (2004)	120	6	Yes
Lenart-Boron (2016)	121	6	Yes
Lestari (2009)	122	1	Yes
Looft (2012)	123	6	No
Lou (1995)	124	1	Yes
Luangtongkum (2006)	125	1	Yes
Mathew (2001)	126	1	No
Mazengia (2014)	127	1	No
Meemken (2009)	128	3	No
Mehboob (2003)	129	1	No
Millar (2007)	130	1	No
Millman (2013)	131	1	No
Miranda (2007)	133	6	No
Miranda (2008a)	134	6	Yes
Miranda (2008b)	137	3	Yes

Study	Reference Number in Original Report	Classification of Intervention(s)	Amenable to Meta-Analysis, Stratified by Intervention Type
Miranda (2008c)	138	3	Yes
Miranda (2009a)	135	3	Yes
Miranda (2009b)	136	3	Yes
Miranda CD (2007)	132	1	No
Mitchell (2004)	139	1	No
Mollenkopf (2014)	140	1	No
Morley (2011)	141	3	Yes
Nannapaneni (2009)	142	2a	Yes
Noormohamed (2014)	143	6	No
Norby (2003)	144	1	No
Nugent (2001)	145	1	No
Nulsen (2008)	146	1	Yes
Nwankwo (2014)	147	5	Yes
Obeng (2012)	150	6	Yes
O’Brien (2012)	148	6	No
O’Neill (2010)	149	3	Yes
Osadebe (2012)	151	6	No
Österberg (2016)	209	3	Yes
Pantosti (1999)	152	5	No
Park (2012)	153	1	No
Patchanee (2008)	154	2b	Yes
Peng (2016)	155	6	Yes
Pettey (2008)	156	1	No
Pol (2007)	157	1	Yes
Price (2005)	158	1	Yes
Price (2007)	159	1	Yes
Ray (2006)	160	1	Yes
Reinstein (2009)	161	1	No
Roesch (2006)	163	3	Yes
Rollo (2010)	164	1	Yes
Rossa (2013)	165	1	Yes
Salaheen (2016)	166	6	Yes
Sanchez (2015)	167	1	Yes
Sapkota (2010)	169	1	Yes
Sapkota (2011)	168	1	Yes
Sapkota (2014)	170	1	Yes
Sato (2004a)	171	1	No
Sato (2004b)	173	1, 3	Yes
Sato (2005)	172	1	Yes
Schmidt (2015)	174	1	No
Schwaiger (2008)	175	3	Yes
Schwaiger (2010)	176	3	Yes
Siemon (2007)	177	6	Yes
Sischo (2010)	178	6	No
Skjøt-Rasmussen (2009)	179	3	No
Smith (1981)	180	2a	Yes
Smith (2013)	181	1	No
Soonthornchaikul (2006)	182	3	No
Sørum (2004)	183	5	Yes
Sørum (2006)	184	5	No
Stegeman (2006)	185	5	No
Struve (2010)	186	3	No
Suriyasathaporn (2010)	187	3	Yes
Tadesse (2009)	188	6	Yes
Tamang (2015)	189	3	Yes

Study	Reference Number in Original Report	Classification of Intervention(s)	Amenable to Meta-Analysis, Stratified by Intervention Type
Teramoto (2016)	190	6	No
Thakur (2005)	191	1	Yes
Tikofsky (2003)	192	3	Yes
Tragesser (2006)	193	2b	Yes
Trost (2013)	194	6	No
Truszczyński (2006)	195	5	No
van den Bogaard (2000)	196	5	Yes
van den Bogaard (2001)	197	6	Yes
Veldman (2014)	198	6	No
Walk (2007)	199	1	No
Wanninger (2016)	210	1, 6	Yes
Warnick (2015)	200	1	No
Wyckoff (2012)	201	1	No
Zawack (2016)	202	6	No
Zhang (2005)	205	6	No
Zhang (2010)	204	6	No
Zhang (2011)	203	1	Yes
Zwonitzer (2016)	206	6	Yes

Supplemental Table 2: Index of human studies, reference numbers, and intervention types

Study	Reference Number in Original Report	Classification of Intervention(s)	Amenable to Meta-Analysis, Stratified by Intervention Type
Borgen (2000)	51	5	Yes
Coalition for animal health (NR)	62	5	No
Cuny (2012)	66	1	Yes
Dorado-García (2015a)	72	6	No
Dorado-García (2015b)	73	6	Yes
Dutil (2010)	74	2b	No
Gallay (2007)	78	2a	No
Harper (2009)	90	6	Yes
Huijbers (2015)	98	3	Yes
Johnson (2007)	100	1	No
Kieke (2006)	107	1	Yes
Klare (1999)	109	5	Yes
Kruse (1999)	111	5	Yes
Kühn (2005)	112	5	Yes
Osadebe (2012)	151	6	No
Rinsky (2013)	162	1	Yes
Skjøt-Rasmussen (2009)	179	3	No
Smith (2013)	181	1	No
Sørum (2006)	184	5	Yes
van den Bogaard (2000)	196	5	Yes
van den Bogaard (2001)	197	6	Yes

2. NARRATIVE LITERATURE REVIEWS

2.1 Illustrative examples of probable transfer of resistance determinants from food-producing animals to humans

Authors	Hattie E. Webb
Institution	Department of Animal and Food Sciences, Texas Tech University, United States of America
Submission	September 2016
Journal publication	Webb HE, Angulo FJ, Granier SA, Scott HM, Loneragan GH. Illustrative examples of probable transfer of resistance determinants from food animals to humans: steptothricins, glycopeptides, and colistin [version 1; referees: awaiting peer review]. F1000Research 2017, 6:1805. doi: 10.12688/f1000research.12777.1 (https://f1000research.com/articles/6-1805/v1).

I. STEPTOTHRICINS

Introduction

Streptothricins are a distinct group of antibiotic compounds isolated from the *Streptomyces* spp.[1,2] The first streptothricin compound (F) was described in 1942.[3] Antibiotic agents of the streptothricin group are composed of varying combinations and proportions of the streptothricin compounds (A, B, C, D, E, F, and X).[4] More than 70 mixtures of streptothricin compounds have been described and subsequently named including streptolin, racemomycin, geomycin, grisein, pleocidin, and nourseothricin; however, the amount of detail available regarding the chemical structure and antibacterial activity of each of the streptothricin antibiotic agents varies greatly. Nonetheless, the streptothricin antibiotic agents are known to be effective against pathogenic fungi and have both bacteriostatic and bactericidal effects on Gram-negative and Gram-positive bacteria through the inhibition of protein synthesis and misreading of genetic information.[5-7]

Usage

Nephrotoxicity associated with streptothricin antibiotic agents has prevented clinical use of these agents in human medicine.[8,9] As a result, use of the streptothricin antibiotic agents has been largely limited to plant production and animal husbandry in a select few countries, particularly China and the former German Democratic Republic (GDR; East Germany).[10,11] The most detailed accounts of streptothricin use and the apparent subsequent dissemination of resistance are available from the GDR. Between 1981 and 1989, nourseothricin—a mixture of streptothricin D and F—was used in the GDR for in-feed growth promotion in the swine industry.[3,12] No data are available about the amounts of streptothricins or nourseothricin produced, distributed, or used in the swine industry during this time. Nourseothricin was not used in animals in the GDR prior to the introduction of its use in swine, and nourseothricin use in the GDR was limited to the swine industry.[13] Furthermore, no use of other streptothricin antibiotic agents in animals or humans has ever been reported in the GDR.

Resistance

It has been indicated that, prior to utilization in the swine industry in 1981, acquired nourseothricin resistance in Enterobacteriaceae among animal and human isolates was rare and believed to be solely associated with chromosomal mutations.[10,14,15] Furthermore, when phenotypic resistance had been reported, it was never found to be a mobilizable resistance, although the extent of antimicrobial surveillance or screening is not cited and is unknown for that period of time. In 1981, less than one year after the initial use of nourseothricin in the swine industry, a streptothricin-streptomycin-spectinomycin resistance phenotype was observed in *Escherichia coli* isolated from rectal swabs from pigs on multiple farms, sewage, and from the feces of those in direct contact with the pigs (i.e. farm personnel).[15] This resistance was found to be mediated by streptothricin-acetyltransferase (*sat*) genes coding for a nourseothricin-inactivating enzyme, which is carried on a transposon, designated Tn1825.[15]

Evidence for transmission

From 1981 to 1983, the plasmid-mediated streptothricin resistance was documented in *E. coli* isolated from rectal swabs of pigs being treated with nourseothricin and slurry from their farms in multiple geographical locations within the GDR.[12] Hummel and colleagues also identified streptothricin resistant *E. coli* in piglets being treated with nourseothricin, the gut flora of persons with direct contact to the pigs (i.e. farm personnel), the gut flora of persons with in-direct contact to the pigs who had no other connection the livestock industry (i.e. farm personnel's family members), and in gut flora of outpatients living in the same region that had no apparent contact with pigs.[15,16] Remarkably, the authors did not observe streptothricin resistance in samples from piglets or humans in regions where nourseothricin was not used. Further, the prevalence of streptothricin resistance was highest in *E. coli* isolated from piglets (33% of 306) and declined in the following order: isolates from farm personnel (18% of 377), isolates from farm personnel's family members (17% of 334), isolates from outpatients (16% of 266) and isolates from urinary tract infections in outpatients (1% of 2).

Despite discontinuation of nourseothricin use in the GDR swine in 1988, the identification of streptothricin resistance and associated resistance determinants continued and broadened. Streptothricin resistance has now been associated with the *sat*, *stat*, and *nat* genes.[17] In 1992, the first report of streptothricin-resistance *Campylobacter* isolated from pig slurry was published.[18,19] Integrons harboring the gene sequence of these resistance determinants have also been observed in other bacteria (clinical isolates, animal environments, and food-producing animals), including *Salmonella enterica, Enterococcus faecium, Acinetobacter baumannii, Burkholderia cenocepacia, Vibro cholerae, Shigella sonnei,* and *S. flexneri*.[10,20-25]

Interestingly, the spread of the streptothricin resistance gene to these other ecological niches and bacterial populations has occurred without direct selection pressure (i.e. use of streptothricins in animals or human medicine).[10] Importantly, the streptothricin resistance genes are often harbored in integrons with resistance determinants present to other antimicrobial agents, namely determinants coding for resistance to streptomycin, spectinomycin, trimethoprim, or kanamycin.[20-23] It is possible that such co-resistance may have contributed to the early dissemination of streptothricin resistance but the early epidemiological studies did not report information on use of other antimicrobial agents. Little to no information is provided about the animals and humans from which the isolates were collected. Furthermore, because there were few studies that searched for streptothricin resistance prior to the 1980s, it is not known if streptothricin resistance determinants were present in bacteria before this time. Nonetheless, this illustrative example outlines the published account of the likely emergence and dissemination of plasmid-borne resistance from swine to humans.

Summary

Nourseothricin, a streptothricin antimicrobial agent, was widely used as a growth promoter in the swine industry in the former German Democratic Republic from 1981-1988. In contrast, toxicity prevented use of streptothricin antimicrobial agents in humans. Less than one year after the introduction of nourseothricin in swine, a plasmid-borne streptothricin resistance (*sat*) seemingly emerged in *E. coli* isolated from swine administered nourseothricin. Subsequently, plasmid-borne streptothricin resistance was detected in the gut flora of humans with direct, in-direct, and no contact to pig farms, but living in the same regions. Following reports of the plasmid-mediated streptothricin resistance demonstrates an illustrative example of the detection—and apparent emergence—of streptothricin resistant bacteria in swine as a result of antimicrobial use, and the dissemination of the resistant bacteria and mobile genetic elements conferring resistance to humans.

II. GLYCOPEPTIDES

Introduction

Glycopeptides are a broad-spectrum antimicrobial class, including vancomycin, and its derivatives teicoplanin, telavancin, dalbavancin, oritavancin, and avoparcin.[26] Glycopeptides block cell wall assembly in Gram-positive bacteria by inhibiting peptidoglycan synthesis.[26] Therefore, the clinical importance of the glycopeptide class has been treatment of infections caused by Gram-positive pathogens. For a large part of the 1980s and 1990s glycopeptides were the drugs of last resort for multidrug resistant Gram-positive infections in humans.[27]

Usage

Vancomycin, the first antibiotic of the glycopeptide class, was first described in 1955 and was subsequently approved for human use by the United States (US) Food and Drug Administration (FDA) in 1958.[27-29] The dates of approval and beginnings of human use in European countries are unknown. Renal toxicity and ototoxicity (largely due to impurities in the drug) limited vancomycin use in humans until the early 1980s when multi-drug resistant Gram-positive bacteria began to emerge and purified formulations of vancomycin became available.[30,31] Annual vancomycin usage in humans in the US climbed from 2,000 kg in 1984 to 11,460 kg in 1994.[31] In Europe and Australia, human vancomycin use was more limited;[31] for example, in Australia, an average of 193 kg of vancomycin was used in humans annually between 1991 and 1993.[32,33] France reported 200 kg of vancomycin was used in humans in 1984, increasing to only 1,151 kg in 1994.[31] Annual vancomycin usage in humans in Germany, Italy, United Kingdom (UK), the Netherlands, and Denmark each ranged between 24 to 408 kg in 1994.[31,34] Human use of vancomycin began to decline after 1994 following efforts to promote vancomycin conservation, an attempted to limit dissemination of glycopeptide-resistant bacteria.

Although vancomycin use in humans in Europe was very limited in the 1990s, avoparcin, a glycopeptide antimicrobial, was heavily used in many European countries and Australia as an antimicrobial growth promoter in livestock.[32] Avoparcin use for growth promotion is documented in Europe as early as 1975;[35,36] and while data supporting heavy use of avoparcin in many European countries is limited, data from Denmark indicate 24,000 kg of active avoparcin were used in swine and broilers in 1994.[34] Austria reported an average of 62,642 kg of avoparcin for animal production use were imported per year from 1992 to 1996.[37] Australia used an annual average of 125,000 kg of avoparcin between 1991 and 1993.[32,33] Avoparcin has never been licensed for use in animals in the US.[38] Following the isolation of glycopeptide resistant bacteria from food-animal products at the retail level, attempts to mitigate the risk of human exposure to GRE through the food chain led to the ban of avoparcin for growth promotion use in Denmark and Norway in 1995, Germany in 1996, followed by the remaining European Union member states in 1997.[18,36,39-43]

Resistance

Transferable glycopeptide resistance in enterococci was first reported in human patients in both France and the UK in 1986, and then in the US in 1987.[44-46] However, it wasn't until the 1990s that considerable attention turned to the evaluation of glycopeptide use and resistance due to differing epidemiological trends between glycopeptide-resistant enterococci (GRE) in the US and Europe. In the US in the 1990s, GRE emerged as a significant cause of healthcare-associated infection and colonization in many hospitals—frequently associated to the high use of vancomycin in those hospitals.[47-49] Hospital-associated GRE infections rose at an endemic rate; with the proportion of vancomycin resistant enterococcal blood isolates climbing from little to no resistance in 1989 to 25.9% in 2000.[36,50] In the 1990s in Europe, prevalence rates of GRE in hospitals remained low; however there were reports of GRE in healthy human carriers in the community (e.g. people with no association to a hospital) and sporadic hospitals outbreaks.[51-53]

Monitoring of antimicrobial resistance to growth promoters was not common practice prior to the mid-1990s.[54] Perhaps as a result, the first detection of GRE isolated from sewage, animals, and healthy humans in the community (i.e. outside of hospitals) were reported in the mid-1990s.[36,39,42,53,55-63] Notably, an association was made between use of avoparcin and the occurrence of GRE in livestock and their environments in Belgium, Denmark, Finland, France, Germany, UK, and the Netherlands—directing a spotlight to food-animal production.[39,47,48,54,57,58,62,64-71]

The differing epidemiological trends in GRE between the US and Europe led to considerable interest to compare GRE from European farm animals fed avoparcin, hospitalized humans, and non-human sources using various molecular methods.[30] Such investigations provided a great deal of insight about the epidemiology of acquired resistance genotypes associated with glycopeptide resistance, particularly the most globally widespread and prevalent glycopeptide resistance in enterococci, vanA resistance. VanA is an inducible resistance to vancomycin and often teicoplanin mediated by a complex cluster of resistance genes (*ORF1, ORF2, vanR, vanS, vanH, vanA, vanX, vanY,* and *vanZ*) often carried on a 10,851 bp transposon designated Tn*1546.*[64,72-74]

Evidence for transmission

Analysis of GRE with vanA resistance revealed a certain level of host-association.[48,75-77] Reports using deoxyribonucleic acid (DNA) sequence typing and phylogenetic analysis for genotyping clustered vanA *Enterococcus faecium* isolates from varying ecological backgrounds into distinct genogroups. Strains collected from pigs and healthy people often clustered together forming a single genotype or cluster. In contrast, isolates collected from poultry and their farmers, veal calves and their farmers, and hospitalized patients from epidemics worldwide each form genetically distinct clusters.[75-77] One of the first insights of genetic relatedness was the observation of a single base change (G8234T) in the *vanX* of Tn*1546*, which was first described by Jensen *et al.*[47,48] The G-variant was associated with isolates collected from poultry and poultry farmers in multiple countries.[48,54,65,78] The T-variant, on the other hand, was predominantly observed in swine isolates from differing countries.[48,77] Interestingly, both G- and T-variants were associated with isolates of human origin.[48] In fact, it was observed that all human samples from a Muslim country—a population that likely eats little or no pork—belong to the G-variant associated with poultry, thus further suggesting GRE transmission may occur between food-animals and humans.[48]

Further investigation of vanA mechanism by Willems *et al.,*[77] revealed amplified fragment length polymorphism (ALFP) genotyping clustered a bank of 255 *E. faecium* isolates from various ecological niches and geographic locations into four genogroups (designated A-D). All isolates collected from pigs and 76% of isolates collected from healthy people clustered to form Genogroup A. Almost all isolates collected from poultry (95%) and 50% of isolates from poultry farmers clustered to form Genogroup B, and Genogroup D contained 70% of isolates collected from veal calves and their farmers. Further, 84% of isolates collected from hospitalized patients from epidemics in the UK, US, and Australia formed a genetically distinct cluster from the healthy humans and animal genogroups, which the authors designated Genogroup C.[77] Similar findings have been demonstrated using various other genotypic methods.[36,69,75,76,79-81]

The VanA gene cluster is now one of many described genotypic determinants encoding glycopeptide resistance and the early genotypic studies described herein only evidence the likely dissemination of a single glycopeptide resistance determinant from animals to healthy people. Further, the differing epidemiological trends between the US and Europe detail two situations that consequently led to the selection of glycopeptide resistance determinants in distinct ecological niches—one in hospitalized patients and the other in healthy humans and animals. Nonetheless, the genetic characterization of the VanA gene cluster provides an illustrative example of the dissemination of glycopeptide resistance from animals to humans following selection due to use of avoparcin for growth promotion.

Summary

Avoparcin appears to have been widely used in food animals, particularly in chickens and pigs, in parts of Europe, since before the mid 1970s. Vancomycin use in humans, in contrast, was very limited in Europe until the late 1990s. It appears likely that the use of avoparcin in food animals selected for the emergence and dissemination of a resistance gene cluster (VanA), which was increasingly identified in animals and healthy people. Molecular subtyping of the VanA gene cluster has identified variants that are more likely to be associated with certain food animal species. Subsequently, GRE were transmitted and found to colonize healthy humans, presumably via the food chain. Therefore, evaluation of the VanA gene cluster variants provides an illustrative example of the probable emergence and selection of a genetic resistance determinant as a consequence of antimicrobial use in food animals, and subsequent dissemination of the resistant bacteria to humans.

III. COLISTIN

Introduction

Polymyxin E (herein simply referred to as colistin) is a cationic, multicomponent lipopeptide antimicrobial agent of the polymyxin family that was first discovered in 1949 and isolated in 1950.[82] Polymyxins are effective against Gram-negative bacilli through their affinity to bind to the positively charged lipopolysaccharide (LPS) of the cell outer membrane.[83] This binding, more specifically to the anionic lipid A of the LPS, leads to disruption of the cell membrane integrity, ultimately leading to leakage induced cell death.[83-85] Two forms of the colistin compound are available for clinical use: colistin sulfate (colistin S) and the pro-drug, colistimethate sodium (aka colistin methanesulfonate sodium, colistin sulfomethate sodium, colistin M).

Usage

The US FDA first approved colistin for human use in 1962—in the form of colistin sulfate; this first approval was for ear drops.[86] The FDA subsequently approved a product for injection—in the form of colistimethate sodium—for human use in 1970.[87] No US data are available on the quantities of colistin used in humans, although use in the US is thought to have been very low as parenteral use in human medicine quickly fell out of favor due to initial reports of nephro- and neurotoxicity.[87-93] More recently, colistin has reemerged as an antimicrobial of interest as a last-resort treatment option for life threatening human infections of multi-drug resistant Gram-negative bacteria, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains.[94-97] Approval dates for human use of colistin products in member states of the EU are not clear; however, it is believed that human use began in the 1960s. More recent estimates of polymyxin consumption in humans are available in the EU/European Economic Area.[98] A sum of 0.8 tonnes of active polymyxin ingredients—including colistin and polymyxin B—were consumed by humans in 22 European countries in 2012.[99] In 2014, polymyxin consumption in humans in Europe was 0.012 defined daily doses (DDD) per 1,000 inhabitants—a 50% increase since the 0.008 DDD per 1,000 inhabitants was reported in 2010.[100] Countries reporting highest use of polymyxin in humans include Greece, Italy, and Slovakia (0.095, 0.025, and 0.025 defined daily doses per 1,000 inhabitants, respectively).[100]

In animals, the extent of colistin sales and use is largely unknown outside of the EU.[101-104] In the US, one colistin product, in the form of an injectable colistimethate sodium, was approved for use in chickens in 1998;[105] however its marketing status is unclear. In the EU, colistin-containing products for use in animals are authorized,[106] though marketing authorization is on a national level and little historical information is available. It is believed that colistin has been used in food-animals in the EU since the 1950s.[98] Colistin is chiefly administered as an oral group treatment in food-producing species to alleviate and prevent Gram-negative infections of the gastrointestinal tract.[102] Such use is predominantly reported in pigs, poultry, cattle, sheep, goats, and rabbits; however, colistin is also used in laying hens and milk-producing cattle, sheep, and goats.[101,102] To date, no data are available that would allow comparison among uses in differing animal species on a European level.

Colistin also appears to be used in food animal production in Asia, although public data are much more scarce. In China, approximately 90% of the 17.5 million tonnes of colistin produced in 2014 were reportedly consumed by the domestic agriculture industry.[103] If so, China likely represents the largest colistin producer and consumer in the world. In comparison, a sum of 545.2 tonnes of active polymyxin ingredients—including colistin and polymyxin B—were consumed by food-producing animals, primarily in poultry and swine, in 22 European countries in 2012.[99] In 2013, polymyxins were estimated to be the fifth most commonly sold antimicrobial class (7%) for food-producing animals across the EU.[102] Reported consumption of colistin in animals varied greatly, ranging from <0.2 tonnes in Slovenia, Sweden, Ireland, and Luxembourg to >100 tonnes in Germany, Italy, and Spain.[99] In another report, annual colistin use in animals in Europe ranged between 0 mg (Finland, Iceland, and Norway) to more than 20 mg (Italy and Spain) per kg of animal biomass.[107]

In March 2015, the European Commission adopted a Decision restricting indications, target species, duration of treatment, and added prudent use warnings to products administer orally to animals that contain colistin as the sole active ingredient.[108] Evidently, such conversations have continued, as the European Commission recently implemented a Directive to withdraw marketing authorizations for all veterinary medicinal products containing colistin in combination with other antimicrobial substances to be administered orally.[109] Furthermore, the European Medicines Agency (EMA) issued a recommendation advising colistin to be used solely as a second line treatment in animals and for sales to be minimized EU-wide.[98]

Resistance

Despite widespread and continuous veterinary use, data gaps persist around colistin resistance. Lack of agreement on standardized *in vitro* screening methods and interpretation criteria has complicated and hindered phenotypic surveillance efforts.[83,110-113] This dilemma is largely a consequence of two important colistin characteristics: a large molecule size—which reduces its rate of diffusion into media—and its affinity to adhere to plastics—which are commonly used in phenotypic methods.[83,113] Until recently, colistin resistance was believed to be extremely rare; however, surveillance efforts were minimal. In fact, mandatory EU monitoring for colistin resistance in *Salmonella* and indicator *E. coli* only began in 2014.[114,115] Even so, many member states have reported technical difficulties in using the only recommended screening method (i.e., broth dilution).[98]

Before November 2015, described phenotypic colistin resistance was associated with chromosomal mutations, which, in theory, would be limited to vertical (clonal) dissemination.[83,116] However, this previous belief was proven too narrow by the description of a novel and highly conjugable plasmid-mediated gene conferring colistin resistance.[103] The gene, designated *mcr-1*, was described in *E. coli* and *Klebsiella pneumoniae* isolated from human clinical isolates, retail meat, and food animals in China, between 2011-2014.[103] The discovery prompted an immediate worldwide response with screening via genomic data mining exercises or else a combination of phenotypic and polymerase chain reaction (PCR)-based methods.[117-123] It has now been retrospectively identified with 100% homology in other members of the Enterobacteriaceae family isolated from human, animal, food, and environmental samples and from multiple continents.[123-130]

Evidence for transmission

In humans, the earliest identified *mcr-1* was found in a *Shigella sonnei* isolate arising from a hospitalized child with diarrhea in Vietnam in 2008.[131] Bacteria harboring *mcr-1* have also been reported in isolates from humans (both infected patients and asymptomatic human carriers) in Argentina,[132] Bahrain,[133] Brazil,[134] Cambodia,[129] Canada,[135,136] China,[103,126,137-144] Denmark,[101,130] Ecuador,[145] Egypt,[146] France,[120] Germany,[134,147,148] Hong Kong,[134,149] India,[150] Italy,[134,151,152] Laos,[120] Malaysia,[134,153] Netherlands,[121,154-156] Norway,[157] Poland,[134,158] Russia,[134] Saudi Arabia,[133] Singapore,[159] South Africa,[160,161] Spain,[134,162] Sweden,[163,164] Switzerland,[165-167] Taiwan,[168] Thailand,[120] United Arab Emirates,[133] UK,[169] US,[134,170-172] Venezuela,[173] and Vietnam.[131] Bacteria harboring the *mcr-1* gene sequence have likewise been documented from food samples on multiple continents [103,119,126,130,135,154,167-169,174-177], suggesting this may be an important route of dissemination from animals to humans.

To date, the earliest identified *mcr-1*-positive isolates are three *E. coli* isolates collected from chickens in China during the 1980s.[178] Interestingly, *mcr-1* has not been detected in isolates arising during the two subsequent decades; however, the reported proportion of *mcr-1*-positive isolates in China begins increasing in 2009.[178] Furthermore, in Europe the earliest *mcr-1*-positive isolate was identified as an *E. coli* originating from a diarrheic veal calf in France in 2005.[122] Observations of *mcr-1* in bacteria isolated from food-producing animals now includes: pigs (Belgium,[179,180] Brazil,[181] China,[182,183] France,[118] Germany,[147,184] Japan,[123,185] Laos,[120] Malaysia,[126,127,153] Spain,[186] Taiwan,[168] Venezuela,[173] Vietnam,[187,188] UK,[189] US[190]), poultry (Algeria,[120,191] Brazil,[181,192] China,[193,194] France,[118] Germany,[184] Italy,[101,195] Malaysia,[126,127,153] Netherlands,[155] South Africa,[196,197] Spain,[186] Taiwan,[168] Tunisia,[167] Vietnam[187]), and cattle (Belgium,[179] Egypt,[198] France,[122,199] Germany,[184] Japan,[123] Netherlands[155]).

Widespread reports of *mcr-1* shortly after its initial characterization indicate the gene was likely being disseminated in an uncharacterized state, and thereby undetected rather than not being present, for a long period of time. The gene has evidently been widely disseminated geographically, as well as across multiple bacterial species of differing origins. Thus far, *mcr-1* has mostly been reported in *E. coli*, although *mcr-1*-positive *Klebsiella*,[103,159] *Shigella*,[131] *Enterobacter*,[137,149] and *Salmonella*[119,125,126,169,175,186,189,194,200] spp. have also been documented. Furthermore, *mcr-1* has been observed in bacteria from wild animals and water samples, indicating the resistance determinant has also disseminated into the environment.[174,201-203]

Retrospective screening for colistin resistant bacteria may be limited by the availability of historical isolates and their genomic data. Further, lack of standardized phenotypic screening methods and the delay in genotypic description have likely lead to the underestimation of colistin resistance; nonetheless, the identification and description of the gene has opened the door for screening via genotypic methods. Nonetheless, resistance is still believed to be rare, particularly in humans and in some regions of the world. The initial paper reported the *mcr-1* gene sequence in 1.4% of 902 *E. coli* and 0.7% of 420 *Klebsiella pneumoniae* clinical isolates in China; however, prevalence among *E. coli* isolates originating from pigs and retail meats in China were surprisingly higher: 20.6% of 804 isolates from pigs at slaughter collected between 2012-14 and 14.9% of 523 isolates from retail meats (chicken and pork) collected between 2011-2014.[103] Still, in the US the *mcr-1* gene sequence is rare. It was detected in one *E. coli* isolate out of 949 animal intestine samples screened and was not detected in more than 44,000 *Salmonella* and 9,000 *E. coli* and *Shigella* isolates from the National Antimicrobial Resistance Monitoring System (NARMS) and National Center for Biotechnology Information (NCBI) genomic database.[190] In many reports to date, phenotypic screening is frequently performed prior to genotypic screening. For example, in France, Perrin-Guyomard and colleagues report *mcr-1* in 0.3% of 590 isolates from healthy pigs in 2011-13, 1.8% of 227 isolates from broilers in 2014, and 5.9% of 239 isolates from turkeys in 2014;[118] importantly, screening for *mcr-1* was performed only on isolates with a colistin minimum inhibitory concentration (MIC) > 2 mg/L. Some limitations are inevitable with this approach, as it implies a level of dependency on the much-debated breakpoints and phenotypic methods.

The technological response afforded by genomics-based methods also is not without limitations. On July 7, 2016, the first account of *mcr-2*, a seemingly distinct gene also conferring colistin resistance was described in *E. coli* isolated from calves and piglets in Belgium.[204] In fact, *mcr-2* appeared to be more prevalent than *mcr-1* among colistin-resistant *E. coli* of porcine origin.[204] Then, on July 11, 2016, the first functional variant of *mcr-1*, designated *mcr-1.2*, was reported in *K. pneumoniae* isolated from a surveillance rectal swab of a child in Italy.[205] There also remain strains that are seemingly phenotypically colistin resistant that do not harbor the heretofore-identified mechanisms associated with resistance.[119,120,131,132,143,179,181,184,188,197,206-212] Very likely, there remain additional yet-to-be-characterized mechanisms of colistin resistance. Much more work is needed to explore other mechanisms of resistance and to fully comprehend the overall prevalence of colistin resistance determinants and their phenotypic characteristics.

Summary

Colistin has been widely used in food animals—particularly poultry and swine—in areas of Europe and Asia for decades, perhaps since the early 1980s or earlier. Colistin use in humans, in contrast, has been extremely limited, at least until recently. It appears highly probable that the use of colistin in food animals has selected for a novel resistance gene (*mcr-1*), identified as far back as the mid-1980s in chickens in China, which has become increasingly identified in isolates from food animals in many regions of the world since its discovery in 2015. This novel resistance gene has more recently been identified among isolates from humans; however, to date *mcr-1* has been more frequently associated with food animal and meat isolates compared to human isolates. These chains of events, despite the data gaps, provide an illustrative example of the probable emergence, selection, and widespread dissemination of a resistance gene as a consequence of antimicrobial use in food animals, and subsequent transfer of bacteria harboring that resistant gene to humans.

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2.2 Biological plausibility of associations between antimicrobial use in food-producing animals and increased risks of human exposures to, and Infections by, antimicrobial resistant zoonotic pathogens

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Summary

This review introduces the knowledge available on molecular mechanisms involved in the emergence and dissemination of antimicrobial resistance (AMR) associated with agricultural uses in the production of livestock, poultry, and aquatic organisms for human consumption. This knowledge provides the scientific basis for the biological plausibility of associations observed between these uses and increased risks of human exposures to and infections by antimicrobial resistant pathogens. For that reason, evaluation of the mechanisms involved in bacterial response to antimicrobial pressure is relevant to an overall assessment of the strength of the evidence in support of interventions to reduce uses of antimicrobials in agriculture and reduce the expansion of AMR and human exposures to AMR pathogens. In addition to animal protein, AM use in agriculture also affects food crops consumed by humans because of the interrelatedness of food derived from animals and crops.

Since the work of Alexander Fleming it has been recognized that all uses of antimicrobials (AMs) contribute to the emergence and dissemination of drug resistance within microbial populations (O’Brien 1997). Because of this, to provide effective guidance for interventions to control AMR, we must address the major uses of AMs, which are in healthcare and agriculture. However, there is still debate as to the relative priority to be given to including agricultural use in national and international programs to combat AMR. This is particularly relevant to the current project to develop guidelines for agricultural use that are consistent with the work on Clinically Important Antibiotics (CIA) within WHO. In compliance with current requirements for guideline development, the Guidelines Development Group on this topic has commissioned useful reviews of the evidence associating AM use in agriculture and exposures of human populations to AMR pathogens, as well as a separate analysis of the information on unintended consequences of restricting AM use in agriculture. Bradford Hill first introduced the concept of biological plausibility (which invokes understanding of mechanisms as first stated by in his paper on causal inference in epidemiology) as integral to evaluations of evidence and decision making in medicine and public health. While mechanism is recognized by WHO as an important element in evaluating evidence for causality in chemical risk assessment (Becker et al 2017), the topic of mechanism has not been considered in developing a dossier of information on the role of agriculture in AMR for the GDG. For that reason, this review is offered for consideration by the GDG.

This paper is not a systematic review, given the time allocated for its preparation. In this paper, some of the most important papers on mechanisms are discussed, with attention to a global perspective and the inclusion of information and reviews on the mechanisms of antimicrobial resistance (AMR) emergence within agricultural contexts as well as its dissemination through multiple pathways to human populations. Because of the interconnectedness of agriculture and human health, particularly through the food supply and the environment, this information is relevant to observational studies that have examined pathways that involve the environment and the food supply. We adopt two perspectives for this review: the One Health paradigm that connects and animal and human health and the microbiome perspective of current microbiology.

This purpose of this short review is to introduce the community of researchers, policymakers, and public health practitioners to the extensive knowledge base on mechanisms that connect agricultural use of AMs and increased risks of human exposures and infections by AMR pathogens through animal:human contact (including occupational settings and geospatial context), the food supply, and the environment.
We begin with a summary of the state of the science on mechanisms of AMR emergence and dissemination in general and with an emphasis on the conditions of AM use in agriculture. In this section we introduce several critical concepts that inform research on mechanisms involved in the emergence and dissemination of AMR: the importance of the microbiome (defined as a complex local or global microbial community, including a broad range of related and unrelated commensal and pathogenic bacterial strains, within a location or a set of interactive relationships), the importance of the food supply as a pathway of transmission from animal to humans, and the role of the environment as the locus of reservoirs or libraries of resistance genes.

In the second section, we review the conditions and contexts of AM use in agriculture. These are well designed for driving selection for resistance through horizontal gene transfer, which is the primary mechanism by which bacteria respond to AM pressure. In addition, the conditions common to intensive food animal production for much of human food – overcrowding, lack of sanitation, administration of drugs without information on target pathogens or AM susceptibility --; are the same risk factors that have long been recognized as major risk factors for AMR in human healthcare settings. Unlike healthcare, there are at present few programs to ameliorate these conditions for most herds and flocks Other conditions of AM use in agriculture – sub MIC exposures; long duration dosing; multiple agents administered in feeds, lack of waste management and lack of biosecurity and biocontainment -- favor emergence, persistence and dissemination of AMR, including MDR phenotypes encoded in mobile gene cassettes. These conditions constitute multiple amplification steps for AMR in agriculture are much greater than those in clinical settings.

We conclude that there is a plausible scientific basis for special and specific concern about agricultural uses of AMs in the increasing severity of AMR in global health. These mechanism-based conclusions add support to the importance of guidance for interventions specific to agricultural AM use as part of the overall WHO program for responding the threat of AMR. This is not to disregard the importance of ensuring prudent use of AMs in healthcare; this paper demonstrates the importance of developing guidance and programs that extent to *all* uses and abuses of AMs.

Our review of mechanisms supports the need for guidance to adopt the One Health model for improving both surveillance and monitoring of agriculture in order to identify critical opportunities for early detection. At present our detection of AMR relies largely upon reports from clinical settings to alert us to the emergence of a new resistance phenotype or resistance genes. There are multiple biases inherent to this strategy such that emergence is usually recognized long after it has appeared within the microbiome. To inform more effective surveillance there is a need for a multi-tiered system of surveillance that includes healthcare, communities, food animals and other biota, the food supply and environmental biosampling. These recommendations are relevant to guidance for developing integrated systems of monitoring and statistically robust systems of data collection. Nonsystematic and non-integrated programs do not support generalization of findings to the national or regional level and or enable linkage of data from agriculture to human populations. The mechanistic perspective of this paper indicates that resistance gene emergence and gene flow are the critical events in AMR.

Much of this selection and gene flow occurs within resistomes (defined as the biological resources available to microbiomes for responding to antibiotic pressure), of which the largest is located within environments external to humans and animals (Perry and Wright 2014). The environmental resistome contributes resistance genes to human pathogens (Finlay et al 2013; Martinez 2008). Thus, mechanistic understanding elevates the importance of analyzing emergent AMRs in environments using state of the art genomics and metagenomics analyses of the microbiome for early detection of emergent AMR prior to the enumeration of human cases.

Introduction

The increasing prevalence and scope of antimicrobial resistance (AMR) has been elevated to the highest level of urgency on the agenda of global health by the UN and many national agencies, all of which have recognized the potentially catastrophic impact of losing the efficacy of drugs that have prevented and treated infectious diseases in millions of people over the past 70 years. Of importance, the 2016 UNGA resolution placed equal emphasis on controlling AM uses in both human medicine and agriculture. However, the role of agricultural use of antimicrobials continues to provoke controversy as to the state of knowledge on the role of agricultural AM use in the overall problem of AMR including lack of scientific consensus on the extent to which agriculture independently contributes to AMR; the factors that drive AMR emergence and dissemination from agricultural sources including food products; and the quantitative contribution of agricultural AM use to the overall burden of human disease related to AMR pathogens.

Mechanistic understanding can contribute to reducing uncertainties related to these questions. The aim of this review is to review the state of knowledge related to the role of agricultural AM use in AMR and the factors that drive AMR emergence and dissemination from agricultural sources to human populations. With respect to the third issue, an understanding of the interrelatedness of agricultural and other sources of AMR can support a re-evaluation of the barriers to assessing burden of disease on a source-specific basis. Many commentaries have concluded that this is not only a difficult question, but it may also be the wrong question (Liebana et al `2013). AMR from agricultural sources is released into the environment by multiple pathways, farmers and workers who are at the front line of exposure, movement of people in and out of communities and healthcare institution along with food products that are consumed by all populations. Consequently, regardless of the original source of AMR, very quickly it becomes difficult to identify and separate agricultural and clinical sources of AMR pathogens that are isolated from human populations. Both genes and pathogens originating in agriculture quickly move into healthcare settings. Equally, strains first defined as healthcare related can move into the human community and into domesticated animals (Price et al).

Determining the original source of AMR is also difficult to resolve. Our knowledge of the time and place of emergent antimicrobial resistance is uncertain and must be tempered by recognition that “emergence” is best defined as “where and when we first notice it”. This information on “where and when” comes almost exclusively from human cases screened in health care settings because most of the resources available for diagnosing infections and detecting resistance are present in health care settings. The focus on infections narrows our surveillance focus to pathogenic strains whereas mechanistic microbiology indicates that commensal organisms within animal and human guts as well as the environment may in fact be the major reservoir of resistance genes in human pathogens (Ravi et al 2015; Shtertzer and Mizrahi 2015; Martinez 2008). These observation biases contribute to the assumption that in terms of AMR selection the most important encounters between bacteria and antimicrobials take place in health care settings (FAO 2016). This may not be the case, and as discussed below less intensive but more extensive encounters in agriculture may more effectively favor selection for and dissemination of AMR. We also fail to recognize the nature of microbiomes as communities as exemplified by a selective focus on AMR in pathogenic organisms. This makes sense in public health and clinical medicine, but it obscures the magnitude and resources of AMR within the microbiome.

Uncertainty about emergence impedes our recognition of non-healthcare associated AMR infections. These are acknowledged only after a significant number of cases are reported without known health care contact. This stage can be prolonged as is the investigation of sources and risk factors outside of healthcare. This has caused delay in focusing on agriculture as a source of non-healthcare association infections. For example, methicillin resistant *Staphylococcus aureus* (MRSA) was first widely reported in hospitals in the 1980s but it took until the mid-2000s for clinicians and public health agencies to recognize that most cases of MRSA were not associated with health care. More years were required for researchers to identify some of the more significant community risk factors, including agriculture and the first reports of livestock associated MRSA were fortuitous. This history alone indicates that the need for a One Health approach that includes both clinical and agricultural uses of AMs as well as the environment, in monitoring of AMR health (Berendonk et al 2015).

Part 1. A primer on molecular drivers and mechanisms of AMR emergence and dissemination.

Almost all currently used antimicrobial molecules are natural products of microbes. AMR has existed for billions of years within and among microbial communities (Perry and Wright 2013). Because of the role of these molecules in the struggle among microbes, resistance was an evolutionary response for survival through natural selection by gene mutation that encoded to traits that conferred resistance to these natural biotoxins through increased efflux or modification of molecular targets. The archaic history of resistance was demonstrated in a recent study of an isolated cave-dwelling bacterium that is resistant to almost all known AMs (Pawlowski et al 2016). In contrast, human uses of AMs are very recent, following on the development of mass production methods in the early 1940s. Because of this history, resistance was already present within the bacterial populations studied by Fleming (Fleming 1944).

During the first years after discovery and deployment of antimicrobials (including clinical and agricultural use), little was known of the drivers and mechanisms for AMR. Evolutionary theory was invoked to explain the emergence of antibiotic resistance as a process of random genetic mutations that was necessary to confer biological resistance to drugs based on selection of organisms with these random genetic changes that enabled resistance to natural agents with specific mechanisms of action, This theory also supported the assumption that each instance of resistance required either vertical transmission from the replication of a resistant organism or a separate evolutionary event. Evolutionary theory also supported the assumption that there was a trade-off between resistance and growth rate (the *r*K selection theory) such that in the absence of AM pressure, microbial populations would revert to being dominated by susceptible strains. Without the “cost of resistance” there would be an even chance of susceptibility or resistance within microbial populations. More recent research has expanded the role of natural selection in AMR and more complex events have been found, such as “bet hedging” by which microbial populations under AM pressure can acquire additional mutations that compensate for the cost of resistance and preserve the resource of resistance genes within the microbiome (Levin et al 2014). These mechanisms explain the persistence of resistant strains in the absence of AM pressure.

Over the past 50 years, a substantial transformation has occurred in our knowledge of the mechanisms of AMR emergence and dissemination. It is now recognized that the major mechanism for the emergence and especially the dissemination of AMR is through horizontal transmission of resistance genes among microbes rather than vertical transmission through cell division or *de novo* mutations. Horizontal or lateral gene transfer (HGT) was observed although not understood mechanistically as early as 1928. Later experiments demonstrated the multiple mechanisms by which susceptible bacterial strains respond *in vitro* to AM pressure through signaling within the microbiome and accepting the transfer of genetic material from one cell to another (Tatum and Lederberg, 1946). Additional studies have demonstrated that resistance genes that are transferred among cells on plasmids and other mobile genetic elements (MGE) can be incorporated into the chromosomal genome of the recipient cell and transcribed for protein synthesis. In contrast to evolutionary mechanisms, HGT enables bacteria (and other microbes) to rapidly respond to antimicrobial pressure through highly efficient community signaling within the microbiome (von Wintersdorff et al 2016). HGT is more active at low levels of AM exposure (Martinez 2008) because of increased signaling among bacteria within the microbiome. This is because higher levels of AM exposure – greater than the MIC – kill organisms whereas sub MIC exposures stimulate signaling pathways that engage horizontal gene transfer events among bacteria (ter Kulle et al 2016).

HGT can move individual resistance genes as well as cassettes of multiple genes that encode for co-resistance and co-selection of resistance within and among microbial communities. Through the process of repeated exposures to multiple AMs, bacteria acquire “genetic capital” in the form of sequential acquisition of resistance genes that can be transferred as a unit through mobile genetic elements (Canton and Ruiz Garbajosa 2011). This process may result in co-selection, in which exposure to any AM represented in the cassette may drive transfer of resistance to other AMs in the cassette. The was first demonstrated in 1989 by experiments showing that cross resistance among antibiotics can be selected by one drug represented in the MDR cassette (Cohen 1989).

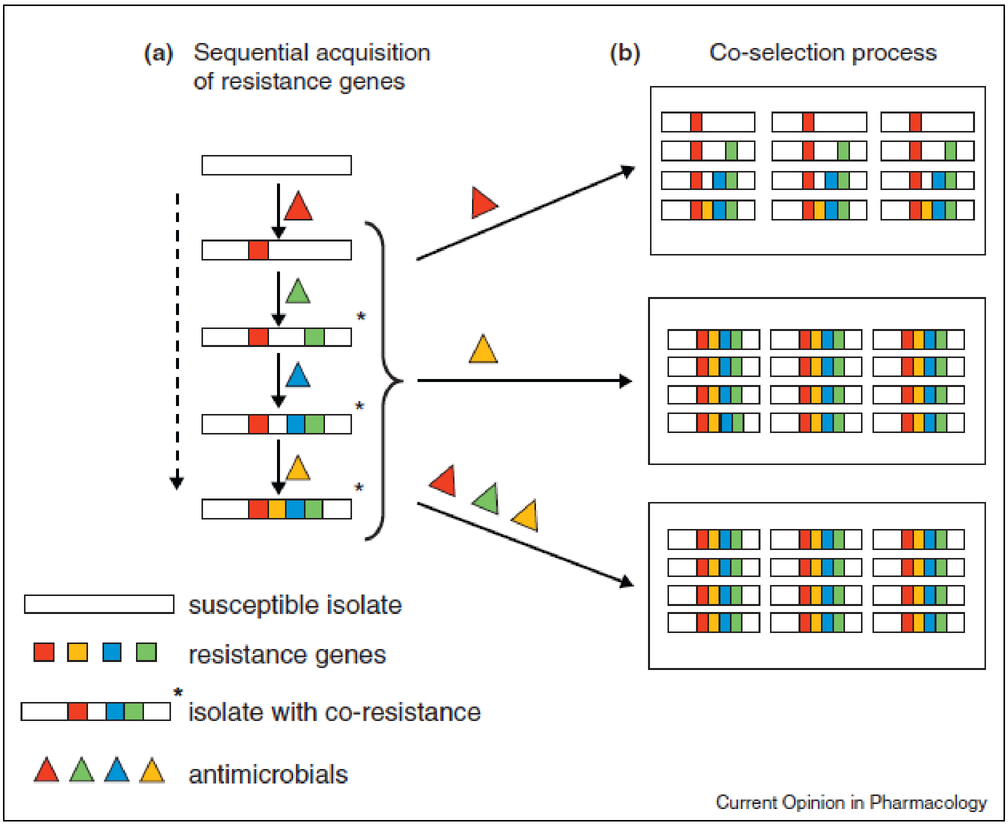

(a) Sequential acquisition of resistance genes
(b) Co-selection process
*
susceptible isolate
resistance genes
isolate with co-resistance
antimicrobials
Current Opinion in Pharmacology

Figure 2. Building genetic capital through sequential acquisition of resistance genes, resulting in facilitation of co-selection through exposure to a single AM (Canton and Ruiz Garbajosa 2011).

These mechanisms indicate the importance of the microbiome perspective, replacing the older concept of bacteria as solitary organisms. We need to expand our concepts the molecular and genomic events involved in AMR by incorporating the perspective of the microbiome. These resources are defined as the resistome (defined as the biological resources available to microbiomes for responding to antibiotic pressure), and the mobilome (defined as the biological resources available to microbes for transferring genes in response to pressure). These are shown below:

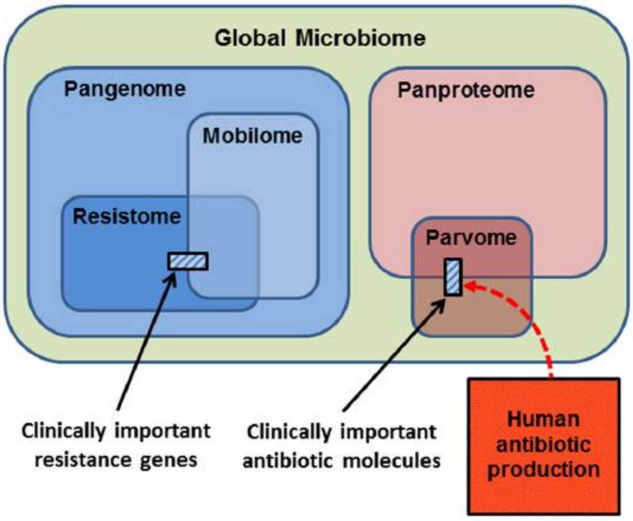

Global Microbiome
Pangenome
Mobilome
Resistome
Panproteome
Parvome
Clinically important resistance genes
Clinically important antibiotic molecules
Human antibiotic production

Fig 3. The relationships within the global microbiome and its pangenome (including the resistome and the mobilome) that support horizontal gene transfer in response to antibiotic pressure including those genes encoding resistance to clinically important antibiotics. The panproteome includes the gene products of the microbiome, including the parvome which includes clinically important antimicrobial molecules produced by humans (from Gillings 2013).

These events take place largely in microbiomes in the environment external to humans and animals, as noted above. For this reason, agriculture is of primary importance, since it is situated with the environment. As shown below, gene flow within food animal production builds the environmental microbiome through multiple pathways.

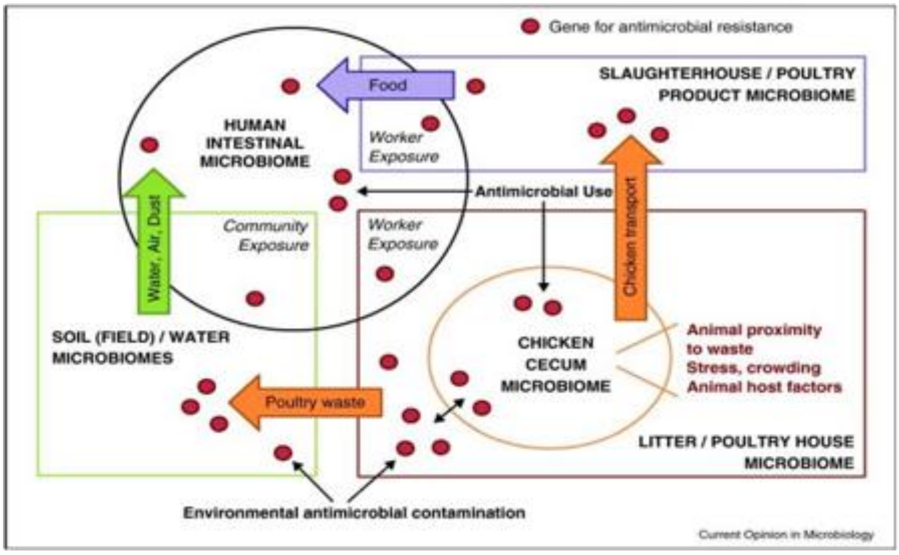

● Gene for antimicrobial resistance
SLAUGHTERHOUSE / POULTRY PRODUCT MICROBIOME
Food
HUMAN INTESTINAL MICROBIOME
Worker Exposure
Antimicrobial Use
Chicken transport
Water, Air, Dust
Community Exposure
Worker Exposure
SOIL (FIELD) / WATER MICROBIOMES
CHICKEN CECUM MICROBIOME
Animal proximity to waste
Stress, crowding
Animal host factors
Poultry waste
LITTER / POULTRY HOUSE MICROBIOME
Environmental antimicrobial contamination
Current Opinion in Microbiology

Figure 4. Gene flow within food animal production, food, and environmental pathways of human exposure (figure from Davis et al 2014).

Empirical assessments of gene flow from agriculture into the environmental microbiome have been made by several investigators (Agerso et al 2000; Nandi et al 2004, You et al 2013). Other studies have documented accumulations of resistance genes as well as AMR bacteria in aquatic sediments in watersheds and estuaries impacted by livestock production and aquaculture (Zhu et al 2017; Muziasari et al 2016). In addition to food contamination, AMR pathogens and AMR genes have been detected in air and wastewater near wet markets (Gao et al 2016).

The response of the microbiome to AM pressure is influenced by the nature of AM exposure. Bacteria are Nietzschean: that which does not kill them makes them strong. Exposures to subtherapeutic concentrations of AMs (or concentrations below the MIC), which are common in food animal production for growth promotion and for some purposes defined as prevention or so-called prophylaxis, are particularly effective as drivers of selection for AMR through increasing growth rates and mutation rates as well as stimulating the transfer of resistance plasmids and conjugative transposons (ter Kuile et al 2016). This seemingly paradoxical observation reflects the fact that higher concentrations of AMs (greater than or equal to the MIC) kill bacteria whereas sublethal exposures stress but spare bacteria stimulate signaling and enhances horizontal gene transfers within the microbiome. Repeated exposures of the microbiome to AMs condition networks of gene flow within the microbiome such that HGT is facilitated (Skippington and Ragan 2011). Continuous or prolonged low level AM use also expands the resistome and further enhances the role of mobile genetic elements in mediating the dissemination of resistance within microbiomes within animal and human hosts as well as the environment. Moreover, exposure to multiple AMs results in accumulation of resistance gene "capital" that drives the emergence and dissemination of multidrug resistance (MDR) though plasmids containing multiple resistance genes that encode resistance to multiple drugs (Canton and Ruiz Garbajosa 2011). Understanding these mechanisms is critical to developing science based guidelines to control and prevent AMR:

Part 2. Food animal production

The brief review above highlights four issues: the difficulty in distinguishing agricultural and nonagricultural sources of resistance, the importance of the microbiome approach to understanding and studying AMR, the dominant role of non-evolutionary mechanisms for AMR resistance and compensatory mutations for conferring AMR persistence, and the effectiveness of low level exposures to AMs in selecting for resistance and driving dissemination.

The first section highlights three types of studies on AMR and agriculture: those in which the first reports of resistance implicated agricultural uses in food production, those in which increases and decreases in AMR in human isolates were associated with additions and bans on AM use in animal feeds, and studies in which AMR was associated with first use of novel molecules in agriculture prior to clinical use. These studies go back nearly 60 years to the early days of intensive food animal production shortly after AMs were approved for agricultural uses (for example, Williams Smith and Crabb1960).

Approval for agricultural use of an AM already in clinical use greatly expanded overall AM use. This has been associated with rapid and substantial increases in AMR in zoonotic pathogens isolated from humans. For example, studies from several countries reported that the prevalence of quinolone resistance in human isolates of *Campylobacter jejeuni* increased dramatically shortly after approval of enrofloxacin, a quinolone drug, for use in poultry feeds.

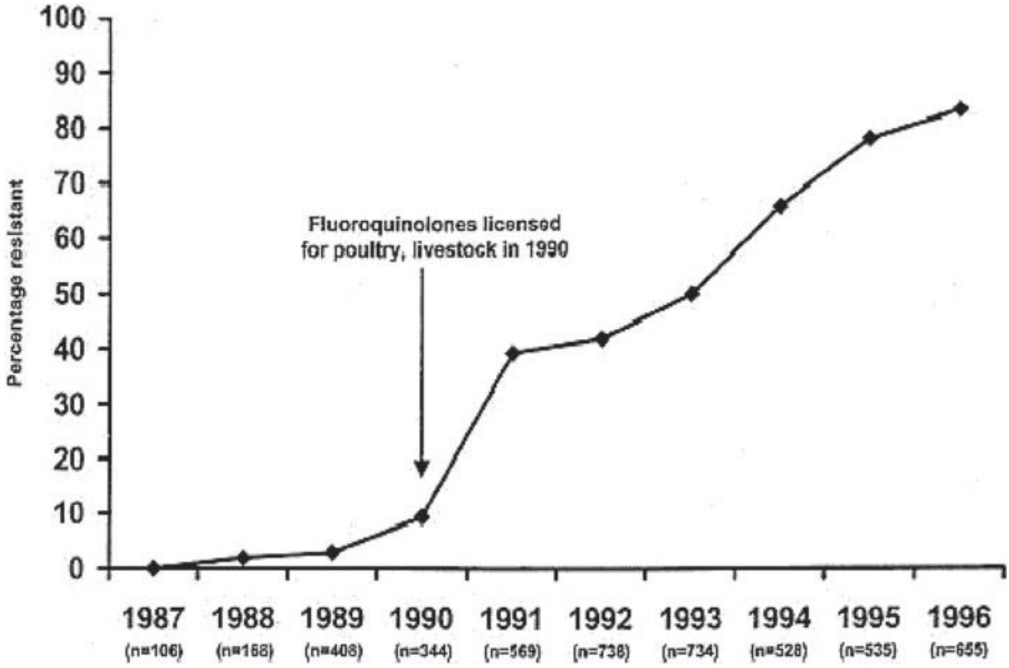


Figure 5. Trends in prevalence of fluoroquinolone resistance in clinical isolates of Campylobacter jejeuni, in Spain, examined for resistance from 1987 to 1996. Before approval of FQs in poultry and livestock production, resistance was relatively rare (<10%); after approval, the prevalence of resistance rose quickly. These and similar data are discussed by Angulo et al, 2004.

There are also examples where increases in exposure to and infections by AMR pathogens in both humans and animals followed approval of a novel AM molecule for use in food animal production prior to approval for its use in clinical medicine. This practice is driven in part by the greater regulatory requirements for information on adverse effects prior to approval of new drugs for clinical uses, which constitutes an economic incentive for the pharmaceutical industry to seek approval for agricultural use to recoup costs of drug development. These first uses can compromise later use in clinical medicine, as predicted by Smith et al (2002) and demonstrated in two cases: quinpristin/dalfopristin (Synercid or virginiamycin) and ceftiofur. Synercid was approved for growth promotion in poultry and swine in 1974 some 25 years before its approval for clinical medicine (Thal and Zervos 1999). Soon after this approval, phenotypic and genotypic resistant strains of *Enterococcus faeciae* were isolated from patients reporting consumption of conventionally raised chicken (Kieke et al 2006). Empirical use of ceftiofur, including off-label non-therapeutic uses, was approved FDA for livestock prior to human use. Reports of rapidly emerging resistance to this class of cephalosporins in human infections caused the FDA to restrict these uses in 2012 (Wittum 2012). These data support the conclusions of the GDG that there should be no use of an antimicrobial on the CIA list that is not currently used in agriculture

This section describes the important differences in conditions of AM use in agriculture and clinical medicine (including veterinary medicine). These differences are relevant to understanding context-specific drivers for selection for emergence and dissemination of AMR.

The most significant overall risk factor driving AMR ***emergence*** in any setting is the volume of drug use. Fleming recognized this risk in his 1945 Nobel Prize speech and it has been well studied in analyses of human AM use. Globally, and within many countries, the largest category of AM use is in agriculture, not healthcare (Van Boeckel et al 2015). These two uses developed in parallel. The use of AMs in agriculture mainly for nontherapeutic (defined as delivering doses below the MIC) purposes began in the 1940s in the US, at about the same time that the same AMs were first produced in large quantities and sold for use in clinical medicine. Over the same time, animal husbandry in many countries was transformed, expanded, and intensified to an intensive industrial model (Silbergeld 2016). Over the past 50 years the industrial model, complete with AM use for growth promotion, has been adopted by many additional countries (FAO OECD 2015). Since then AM use in agriculture has steadily increased with most of the same mechanistic classes of AMs used in both clinical medicine and food animal production. This is important to stress: while in some cases different molecules have been utilized in agriculture, such as avoparcin (a substitute for vancomycin) and enrofloxacin (a substitute for ciprofloxacin), these drugs share the same mechanistic targets and thus they exert the same pressure for driving resistance. In most countries, data on AM use in agriculture is considerably less available than data on clinical sales or use. In a few countries, national reporting systems provide detailed information on specific agricultural uses such as growth promotion, prophylaxis, or treatment (Hammerum et al 2007). Other information is based on estimates inferred from data on animal production and specified AM uses (e.g., Krishnaswamy et al 2015). However, some of these uses may overlap because of unclear definitions (You et al 2015). Moreover, these estimated values are likely to be low since they do not reflect the reality of drug use including the lack of veterinary supervision in many countries (Wittum 2012; Roess et al 2015). Even in countries with more controls on AM uses in agriculture, there is some imprecision in defining these uses as well as restrictions on use In some countries, AMs are no longer approved or recommended as feed additives for growth promotion but the same subtherapeutic concentrations originally approved for this use have been redefined as use for prophylaxis (You and Silbergeld 2014).

For agricultural use, there are additional data relevant to testing associations between agricultural use and AMR in food and humans. In the spirit of Koch’s postulates, we can test associations between use and resistance based on recent changes in policy in certain countries. Bans on the use of specific AMs as growth promoters in hogs and poultry in some countries have been followed by significant reductions in the prevalence of resistance to these same AMs in *Enterococci* isolated from animals, food products, and human subjects in those same countries (Hammerum et al 2007).

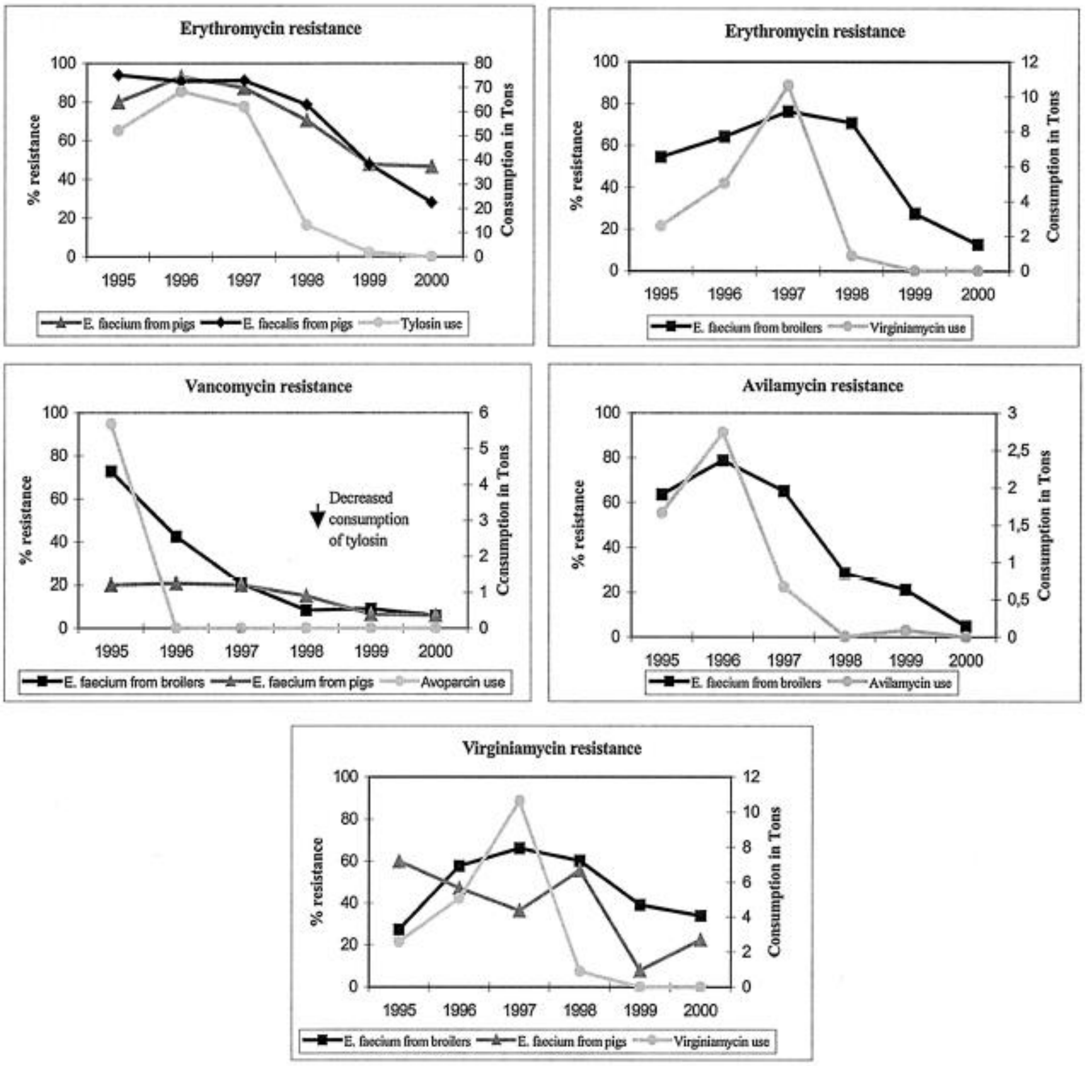


FIG. 1. Consumption of antimicrobial agents for growth promotion and occurrence of antimicrobial resistance in *E. faecium* and *E. faecalis* isolates from Danish pigs or broilers from 1995 to 2000.

Fig 3. Consumption of AMs for growth promotion in Danish pigs or poultry and prevalence of AMR in *Enterococci* isolates from animals (Hammerum, et al. 2007)

The most significant risk factors for AMR ***dissemination*** from animals to humans are the pathways involving food and the environment, each invoking a multiplicity of amplifications that occur throughout the process of food production from "farm to fork" (Silbergeld et al 2008; Berendonk et al 2015). In addition to animals, this includes human food derived from plants that are contaminated by AMR pathogens through environmental pathways of water and soils that in turn have been contaminated by AMR from releases from food animal production into air and soils. This is largely related to the use and disposal of animal and production house wastes. There are numerous studies of actual food borne disease outbreaks and other exposure from contaminated food crops that are consumed by humans, in which contamination by AMR pathogens resulted from use of animal wastes as manures (as is recommended in organic production guidance) or from irrigation by surface water sources contaminated by run off from land disposal of animal wastes (van Hoek et al 2014; Ben Said et al 2015).

The food supply and the environment connect healthcare and agriculture. The same sources of food are eaten inside and outside of healthcare facilities and hospitals are in environments with sources of air and water that may be contaminated by agricultural releases. In addition, people – patients, visitors, and healthcare personnel -- move in and out of healthcare settings (Silbergeld et al 2008). For this reason, there are no real barriers between the presence of AMR in agriculture and the entrance of these same AMR pathogens into healthcare settings. This traffic goes both ways. The cross transmission of so-called "livestock" strains of MRSA (ST398) from humans to animals and from animals to humans has been documented by genomic analysis (Price et al, 2012). As noted above, these interactions make it difficult, if not impossible, to define and quantify agricultural and health care related attributable risks of human exposure to AMR pathogens.

Because of the lack of familiarity of many persons involved in programs of AMR control with current methods of production in intensive food animal production systems that are rapidly supplanting small holder operations, the next section describes these conditions as risk factors for AMR. These factors include conditions on the farm and in slaughter and processing in addition to the conditions of AM use in agriculture.

Many of these are like those that have been identified as risk factors in healthcare setting for which interventions and guidance programs have been developed.

Subtherapeutic and extended exposures of animal gut microbiota and other microbiomes – National registration requirements for AM use in agriculture generally stipulate that low (sub MIC) concentrations are to be used in animal feeds for growth promotion and in many cases because of blurred definitions also for purported prophylaxis. In contrast, in human and veterinary medicine, the dosages of AMs utilized for treatment *and* prevention are designed to deliver doses sufficient to kill targeted pathogens. These low dosages were established first by the US FDA in the late 1940s to avoid drug residues in edible tissues and they have been generally adopted by other national regulatory bodies. In addition, unlike most uses of AMs in healthcare, which are generally limited to a few weeks, food animals can be exposed to AMs in feeds for extended periods of time (weeks or months depending on species and production methods).

Co-exposures to drug mixtures and other agents in feeds - Regulatory documents and registrations for AM use in agriculture, compendia of AM use, as well as the scientific literature, indicate that in many instances multiple AMs are added to animal feeds at the same time, especially for growth promotion, resulting in multidrug exposures of the animal dermal and gut microbiomes (Shturtzer and Mizrahi 2015) as well as those microbial communities in animal wastes into which AMs are spilled or excreted unmetabolized. These patterns of use also differ from most AM uses in healthcare. Multidrug exposures contribute to driving multidrug resistance in isolates from agricultural settings as well as food products (ter Kuile et al 2016; Johnson et al 2016). In addition, the presence of metals (cadmium, zinc, mercury, and arsenic) as trace elements or contaminants in animal feeds can drive both emergence and dissemination of AMR as well as virulence (Argudin, et al 2016; Hinchliffe et al 2016). Cadmium and zinc are added to feeds; mercury is a contaminant that can be present in fish meals often added to animal feeds: arsenicals are used separately as coccidiostats. In some countries, industrial waste streams containing these metals are permitted as additives to animal feeds (reviewed by Sapkota et al 2007).

Movement of animals and food In many regions, there is extensive regional and international movement of live animals at different life stages over the course of production. This has been associated with the spread of avian influenzas, porcine viruses, and other zoonotic infections. Animal movement can also result in transfers of bacterial resistance genes and resistant organisms across national boundaries. Osman et al (2014) reported on higher prevalence of antibiotic resistant strains of *Salmonella typhimurium* carried by turkey poults (a technical term for young turkeys) imported into Egypt as compared to poults of domestic origin. Agersø et al (2014) reported extensive prevalence of ESBL+ resistance to fourth generation extended spectrum cephalosporins in *E coli* isolates from poultry in Denmark despite lack of use of these AMs in Danish poultry productions. The likely source was determined to be imports of chicks from Scotland, where these drugs were permitted for use in food animal production until 2012.

The global market for food products from animals is much more extensive, varying widely among countries and by food item. For many countries, the largest import items from the global marketplace are fish and crustacea often produced in impoundments with the use of AMs (reviewed by Gormaz et al 2014). Pork, beef, and poultry products are extensively exported by the major producing countries (FAO 2016). The volume of these and other food imports is such that most countries are not able to carry out comprehensive assessments of food borne pathogens in these products within the time frame for perishable items clearing customs.

Because of this, we have no readily available information on comparative rates of pathogen carriage in domestic and imported foods.

Production methods in agriculture   Many of the conditions that drive emergence and dissemination of AMR in agriculture have long been recognized as risks for increasing disease emergence and transmission in healthcare settings. These include lack of sanitation, overcrowding of patients (animals), and failure to determine the pathogen associated with a presenting infection as well as its susceptibility to AMR treatment. While programs are mandated in many countries to improve these conditions in healthcare, these remain the customary conditions under which young animals are produced and food animals are raised (You et al 2015). These are discussed below.

Housing In intensive food animal production, animals are confined to buildings or feedlots in very crowded conditions. They share food and water sources and are housed for the duration of their lives amidst their wastes (poultry) or on slatted floors above open cesspits (hogs). Some aquaculture operations grow fish and crustacea in artificial impoundments where they are exposed to antimicrobials as well as their own wastes containing antibiotics and bacteria (Gormaz et al 2014). Cattle are held in feedlots or milking stanchions where they defecate. These "confinements" are not confined and they are not always routinely cleaned between groups of animals such that AMR may persist from herd to herd and flock to flock via contaminated bedding material and waste pits.

*Physiological stress***.** The housing and operating conditions described above induce stress in animals resulting in depressed immune function, increased pathogen infection and carriage, and increased pathogen shedding. In a study of pigs, stress was the most significant factor in increasing the prevalence of infection within a herd such that by the end of the growing period, almost all hogs were carrying the same zoonotic pathogens (Berryman et al 2013). Similar results have been reported for poultry (Hayes et al 2004). These stresses continue through the stages of transport and holding at slaughter and processing plants.

*Environmental releases***.** The buildings or impoundments (feedlots or ponds) used in intensive food animal production are called "confined" but they are not truly bio contained or bio secured (Leibler et al 2016). There are at least three breaches due to operational requirements: ventilation, waste disposal, and influx and efflux of humans, insects, and other pests. Ventilation is essential for modern animal facilities designed to house up to 200,000 chickens and tens of thousands of hogs to prevent animal deaths and support optimal growth. Because of the high density of suspended particulates, filters cannot be used and pathogens from animal houses have been recovered up to 500 m downwind from ventilation fans. While human movement can be managed, it is not possible to prevent movement of insects and other biota in and out of housing.

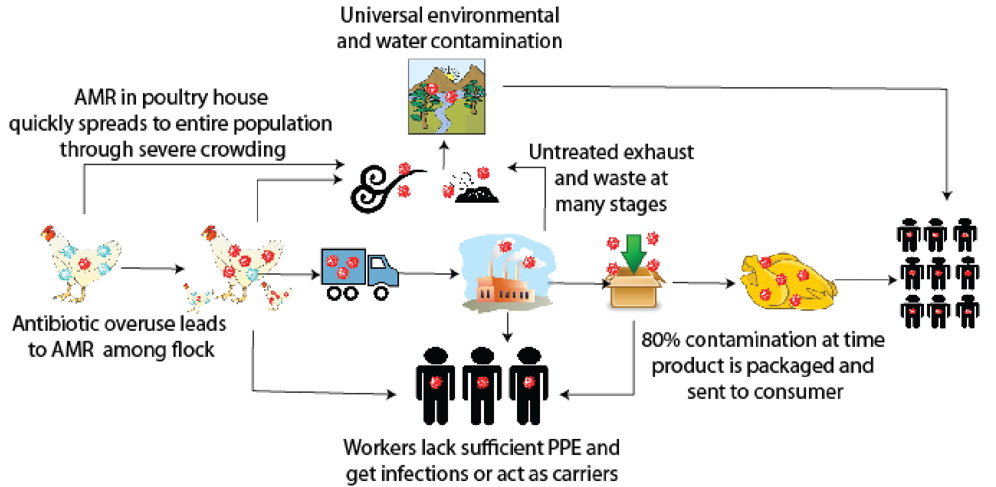
Waste management. Animal wastes have traditionally served the agricultural cycle through use as fertilizers. However, the high stocking density of intensive food animal production operations (number of animals per house) as well as the intensity of geographic consolidation of these facilities (numbers of houses per unit area) results in volumes of waste that often exceed local and even regional carrying capacities such that land application is more accurately defined as disposal rather than use. These wastes also differ from animal manures of traditional animal husbandry: they contain AMR bacteria, resistance genes, other constituents of feeds (including metals) as well as antimicrobials spilled from feeders or excreted unmetabolized by animals. In most countries, there are no requirements for treating these wastes prior to land amendment or disposal. Holding times and composting are recommended by some national agencies prior to use for soil amendment but there is little evidence that these recommendations reduce pathogens or other pollutants in wastes. As a result, the land disposal of animal wastes is a major source of environmental releases to soils and water. Incremental loadings of soils with antibiotics, antimicrobial resistance genes, mobile genetic elements, and AMR pathogens have all been described (You and Silbergeld 2014). Martinez (2008) has aptly described this transfer of resistance genes as environmental pollution.

*Worker exposures in food animal production***.** Unlike healthcare, where occupational safety and health agencies have established regulations and good practice guidelines to prevent exposures of persons employed in healthcare as well as transfers within and from the workplace, there are no regulations related to occupational exposures to pathogens in food animal production (Castillo Neyra et al 2015). There is no routine surveillance or monitoring of workers for these exposures. Many studies have documented that farmers and workers in animal slaughter and processing are exposed to AMR pathogens associated with work. In situations with inadequate protective equipment or resources for worker hygiene, these pathogens can be transferred to communities and households through fomite transfer on contaminated clothing, shoes, and other surfaces. A large study of slaughter and processing plant workers reported associations between work involving close contact with animals and use of sharp tools with increase prevalence of skin and other Infections (Kyeremateng Amoah et al 2015). Risks of. exposure and infections have also been documented in workers' families and household members (as early as 1960 by Williams Smith et al 1960, and later by Fey et al 2000 and Rinsky et al 2013).

*Dissemination through other biota***.** Interactions between humans and domesticated animals are not the sole biotic route of dissemination of resistant zoonotic pathogens from animals to humans. The lack of biosecurity of animal production facilities extends to lack of control over the entrance and exit of other animals, including insects, small birds and rodents. These intruders are attracted by food within the houses (feces for synanthropic flies; spilled grains for birds and rodents). Flies trapped near poultry houses have been found to carry pathogens like those in the houses and in poultry house wastes that are customarily stored in the open or under a roof (Nazni et al 2005). Small rodents enter and leave poultry and swine houses carrying pathogens, including livestock associated MRSA, into the environment (Backhans and Fellstrom 2012). Transfers of AMR bacteria from domesticated to wild avians are well documented in the literature and, in addition to avian influenzas, there are reports on the transmission of ESBL-producing bacteria and ESBL genes between farm animals and wild avians (Bonnedahl et al 2000). These transfers are completely uncontrolled in so-called integrated operations in which chickens are raised over fish ponds where waterfowl are also raised (Leibler et al 2006).

A summary of this discussion is shown below as a series of amplifications over the food production process from farm to fork.

(a) Agricultural AMR Transmission


Universal environmental and water contamination
AMR in poultry house quickly spreads to entire population through severe crowding
Untreated exhaust and waste at many stages
Antibiotic overuse leads to AMR among flock
80% contamination at time product is packaged and sent to consumer
Workers lack sufficient PPE and get infections or act as carriers

(b) Hospital AMR Transmission

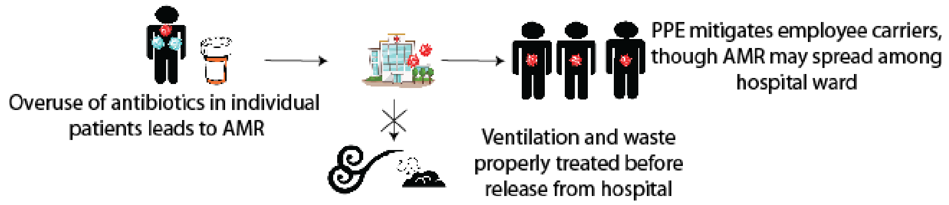

Overuse of antibiotics in individual patients leads to AMR
PPE mitigates employee carriers, though AMR may spread among hospital ward
Ventilation and waste properly treated before release from hospital

Fig 6. Schematic representation of amplification steps in agriculture and health care.

The relative importance of the conditions of AM use in agriculture may be compared to healthcare in terms of a series of amplifying steps in dissemination. Prior to the first step in amplification, there is a larger volume of use or input of AMs in agriculture as compared to healthcare in most countries as discussed earlier. In the first step of Figure 6, the population of animals exposed to AMs within one confinement area is between 20-200,000 chickens and 2 to 10,000 hogs (depending upon life stage and size of the operation). In many countries, large numbers of these confinement houses are located within a restricted area. This is considerably different from the one on one encounter between two humans (health care provider and patient) or the population and number of out- or inpatients at health care institutions within a comparable geographic area. The conditions of housing – overcrowding and lack of sanitation – that are standard practice in poultry and hog production, facilitate transmission of pathogens among all animals within a house. In contrast healthcare facilities are generally required to practice extensive precautions to prevent widespread dissemination of infectious agents with an emphasis on drug resistant pathogens. While many healthcare institutions have substandard conditions, they are usually smaller operations in under resourced areas or emergency situations. The extent of releases from animal confinement houses is very large through air release and waste disposal. This may also occur in healthcare facilities, but in many countries, these wastes are required to be treated prior to discharge and ventilation is controlled. Because of the larger populations in animal production, the scale of environmental releases is considerably greater that those from healthcare facilities. As noted above the volume of food animal wastes exceeds that of human wastes in many regions of intensive food animal production. These environmental releases can result in contamination of crops. Chickens and hogs are transferred to slaughter and processing plants in open cages from which pathogens are released. These plants may process between 1000 and 25,000 chickens and as many as 35,000 hogs per day. Conditions at these plants result in cross-contamination among animals prior to slaughter and among carcasses and food product during processing (Silbergeld 2016). These slaughter and processing plants may also release pathogens into water through their waste discharges. Finally, the global consumption of food products from animals, particularly poultry, is another stage of amplification as well as dissemination that continues to increase (Silbergeld 2016).

Part 3. ESBL mediated resistance in Enterobacteriaceae: a case study of mechanisms of AMR in agriculture

The last part of this review analyzes mechanisms of resistance as these have been related to the emergence of extended beta-lactamase (ESBL) mediated resistance in *Enterobacteriaceae.* This is an urgent and informative example of the role of agriculture in AMR. ESBL refers to extended spectrum beta-lactamases, which are a family of bacterial enzymes that confer resistance in *Enterobacteriaceae* to a broad set of beta-lactam antimicrobials including penicillins and cephalosporins. ESBLs are encoded by several gene classes, including metallo-beta-lactamases (class B) and carbapenemase (class D). ESBL mediated resistance is not a new concern. In 1940, early in the antibiotic era, bacteria were observed to be capable of resisting penicillin exposure by degrading the drug *in vitro*. This observation was an early signal of the resources present within the microbiome for responding to future anthropogenic uses of natural products, prior to our understanding of the mechanisms of antibiotic resistance.

ESBL+ and carbapenemase-producing microbes are present in human and animal hosts (both domesticated and feral) as well as the environment. ESBL+ positive organisms include both pathogenic and commensal strains of *E coli* and other bacteria. As recognized by WHO, ESBL+ pathogens are significant risks for human health, with increasing global prevalence, and the drugs available to effectively treat these infections are classified as critical antimicrobials. For patients infected by ESBL+ pathogens, risks are increased for severe morbidity and mortality, as well as the costs of medical treatment (Tamma et al 2011).
Much of the recent research on this topic incorporates mechanistic studies, using the power of analyses that apply advanced genomics to molecular microbiology. These methods provide rigorous testing of the livestock origins of specific AMR pathogens as well as strains capable of spreading AMR (including ESBL genes) within the microbiome (Skurnik et al 2015).

Penicillins and cephalosporins are among the first and still most widely used antimicrobial drugs to treat infections by gram negative pathogens. Because of the prevalence of resistant *Enterobacteriaceae* infections, these drugs remain critically important for the prevention and treatment of several high priority pathogens including pathogenic strains of *E coli* and *Klebsiella.* (e.g., CDC 2013 http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.) The carbapenem antibiotics were introduced in 1985 to treat infections resistant to the older β-lactams. Resistance to the carbapenems is now prevalent (Molton et al 2013). Resistant strains of many of highly pathogenic bacteria have now been reported world-wide from the Americas, Europe, Asia-Pacific, and Africa. Carbapenemase-producing strains of *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* and other pathogens are also increasingly detected in human populations (CDDEP 2016).

Similar to the temporal trajectory of other important resistance traits, an epidemiological shift has occurred for infections by ESBL+ E *coli* and other pathogens such that ESBL resistance is now increasingly associated with exposures outside healthcare (often referred to as community associated infections). As usual, there is considerably less information on risk factors for non-health care associated infections, but food consumption is recognized as an important factor (Liebana et al 2012: Greko et al 2009).
Since the discovery and exploitation of AMs as natural products, we have experienced increasingly shorter periods of drug efficacy between the introduction of a new drug and the emergence of drug resistance. In the case of ESBLs, as newer cephalosporins were identified and introduced into use over the past 6 decades, beta-lactamases with extended spectra of inactivation have quickly emerged and their resistance genes have been widely disseminated within the global microbiome (Shigemura et al 2011).

ESBL+ resistance largely spreads by the highly efficient mechanisms of horizontal gene transfer. As with other AMR traits and genes, once resistance to a novel cephalosporin has emerged, both resistance genes and resistant bacteria have spread rapidly and globally largely through HGT. To an increasing extent, ESBL resistance is coupled with other resistance determinants that are packaged in gene cassettes and transferred as cassettes among bacteria by mobile genetic elements. Most recently, the combination of ESBL and polymixin resistance has been reported in agricultural settings and animals in many countries (Skurnik et al 2015). This packaging process is enhanced by the complexity and conditions of on-farm exposures to AMs and other co-factors present in agriculture as discussed above. The simultaneous horizontal flow of multiple resistance determinants favors the emergence and persistence of multidrug resistant organisms in agricultural settings that have been reported in many studies. Moreover, through mechanisms of co-selection multiple resistance genes can be horizontally exchanged within microbiomes such that resistance to drugs not being used at the time can result from exposure to any of the drugs represented in the cassette. This has been suggested for the rapidity of selection for resistance to novel cephalosporins as well as colistin (Mollencamp et al 2016).

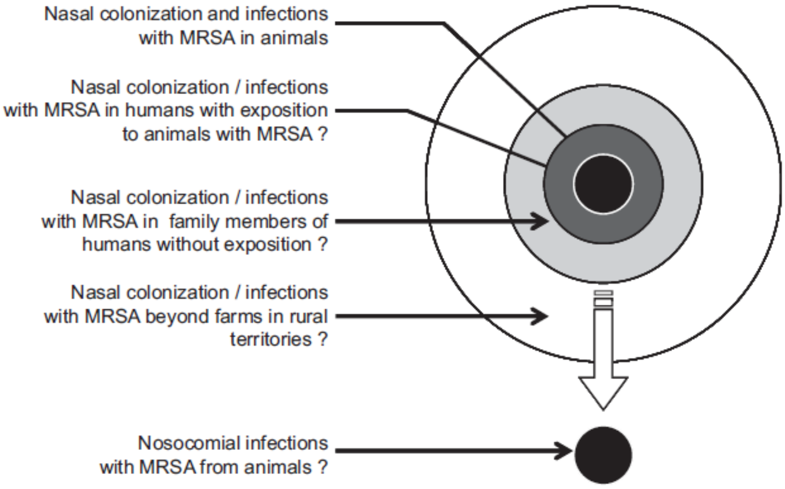
ESBL resistance is a significant challenge in healthcare settings, but in line with the focus of this review, this paper will emphasize agriculture and food as sources and drivers for ESBL resistance emergence and spread. Penicillin was one of the first antibiotics to be approved for growth promotion in food animal production (Silbergeld 2016). Current uses of penicillins in agriculture have been described by region by van Boeckel (2015). Krishnaswamy et al (2015) estimated current use of penicillins in poultry and swine production in China.

Cephalosporins were introduced into agricultural use later than the macrolides. Denmark restricted the use of these drugs for treatment in swine production in 2009 (DANMAP 2015). However, relatively less information is available on volume of cephalosporin use in other countries. Despite the concerns of medical and public health experts, the newest drugs in this class have continued to be approved for empirical as well as designated use or used without formal approval in food animal production. Cephalosporins -- including cephadexin, cefapirin, cefazolin, cefotaxime (third generation), ceftiofur (third generation), and cefquinome (fourth generation) -- have all been approved for agricultural use in the EU (Greko et al 2009). Through HGT mechanisms, resistance to each new drug has been counteracted by new resistance genes. A longitudinal study of archived soil samples found increasing presence and diversity of ESBL+ genes from 1969 to 1974 (Graham et al 2016).

Although carbapenems are not generally registered for use in agriculture, as noted below their use in food animal production has been reported from some countries. Fewer data are available on agricultural uses of these AMs in developing countries. Based on the rapid adoption of practices from highly developed countries, it is likely that these drugs are now widely used in food animal production in many countries. There is very little information on use by smaller production systems, A recent study of smallholders in Peru reported on resistance to cephalosporins and many other antimicrobials in *E coli* isolates from poultry and farmers (Braykov et al 2016).

Carbapenemase is less frequently reported in bacteria isolated from food but it is known to be present in agricultural settings and in bacteria carried by food animals and other biota. Moreover, the use of extended spectrum cephalosporins, common in agriculture, may be a source of co-selection pressure for carbapenemase mediated resistance (Mollenkamp et al 2016).

Cuny has proposed an informative schematic to demonstrate how the use of antimicrobials in agriculture contributes to the emergence of drug resistance (and eventual spread from animals to humans (Cuny et al 20016). This example refers to MRSA, but it is highly relevant to discussions of the steps in other AMR resistance phenotype, including ESBL, within agricultural settings and regions (Michaels et al 2015).


Nasal colonization and infections with MRSA in animals
Nasal colonization / infections with MRSA in humans with exposition to animals with MRSA ?
Nasal colonization / infections with MRSA in family members of humans without exposition ?
Nasal colonization / infections with MRSA beyond farms in rural territories ?
Nosocomial infections with MRSA from animals ?

This schematic identifies animal hosts as the first sentinels of the presence of resistance in zoonotic pathogens in food production; these infections then spread through adaptation of strains capable of infecting humans to farmers and other workers on farms, then to family members on farms, and from farm households to other populations in agricultural regions.

There is evidence for these steps of transmission of ESBL+ *E coli* from farm animals to farm workers and farm families related to use of extended spectrum cephalosporins on the farm. Fey et al (2000) reported on a case of ceftriaxone resistant salmonella infection in a child living on a farm. This isolate was resistant to 13 antimicrobial agents and all but one of the genes encoding resistance were on the same plasmid with the gene encoding 1 b-lactamase CMY-2. Studies of livestock associated MRSA have demonstrated that resistance originating in food animal production can spread to persons with no direct contact with animals or farms; the risks of these exposures are related to the density of animals within the region and likely involve environmental pathways (Feingold et al 2012).

Contamination of the food supply is the step of greatest importance for the direct dissemination of resistant zoonotic pathogens from agriculture to human populations on the local, regional and even global scale. However, because there are no national programs currently capable of assessing ESBL+ pathogens in the food supply, we cannot estimate the current magnitude of this route of exposure. There are studies from several regions that have reported on ESBL genes and resistance in pathogens isolated from poultry and other food animals, as well as from food products and within the environment of animal slaughter and processing plants. There are also studies documenting temporal increases in the prevalence of ESBL-producing strains of E coli isolated from food animals. Limited evidence on increasing temporal trends in ceftriaxone resistance were reported by the US NARMS (2012-2013 report) in isolates from humans and cattle from 2000 to 2013 (Frye and Fedorka-Cray 2007).

The prevalence of ESBL-producing *E coli* isolated from the guts of food producing animals has also increased from 3% in 2003, to 15% in 2009, to 100% in 2009 in a study conducted in the Netherlands (Leverstein-Hall et al 2013). Leverstein-Hall et al (2011) utilized ESBL specific sequencing to demonstrate transfers from live poultry to consumer poultry products and to humans. Overdevest et al (2011) undertook a community based study in which isolates from locally sold consumer poultry and other meat products were matched to cultures from rectal swabs and blood isolates from persons in local hospitals. Of the chicken meat samples, 80% of the *E coli* isolates tested positive for ESBL, with the predominant genotype being *bla-CTX-M1*. There was a high degree of similarity, by MLST analysis, between the *bla-CTX-M1* genes from chicken isolates and human rectal swab isolates with less similarity between poultry isolates and human blood cultures.

In summary, the mechanistic lessons we have learned from fundamental microbiology as well as the analyses of drug- and. pathogen-specific instances of AMR associated with different agricultural uses of AMs provide strong support for the biological plausibility of associations between agricultural use of AMs for all purposes and increased risks of AMR exposure of animals, food, workers, communities, human populations, and the environment. These linkages and mechanisms may provide insights for new methods of early detection in prevention the emergence and global dissemination of AMR. Mechanism indicates that our focus should be expanded to gene flow with the application of state of the science methods of next generation genomic and metagenomic sequencing that can support observational studies and define the opportunities to reduce the contribution of agriculture to the global AMR crisis more precisely. Gene flow also indicates that is not useful to attempt to allocate the contribution of agriculture and healthcare to the overall burden of AMR. While genes flow across the microbiomes of humans, animals, and the environment, the expanded microbiome largely resides in the environment.

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2.3 Potential unintended consequences associated with restrictions on antimicrobial use in food-producing animals

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Executive Summary

Conclusions

- Overall, the adverse consequences of AGP bans and other restrictions described in the literature appear to be limited and temporary.
- Based on European experiences with terminating AGPs, such adverse effects that may be encountered can be reduced by taking steps to minimize disease in vulnerable classes of animals, especially weaner pigs, and supporting producers in making a transition to more targeted, prudent antimicrobial use. Such steps include improvements in veterinary advice, animal housing, non-antimicrobial disease control strategies and antimicrobial use surveillance.
- For future AGP bans, particular care is needed to avoid compensatory increases in antimicrobial use for disease prophylactic or therapeutic purposes, particularly antimicrobials important for therapy in either humans or animals.

Restrictions on the use of antimicrobials in food-producing animals generally seek to reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistance in humans and / or animals. The purpose of this review, however, is to summarize the evidence for effects of these restrictions on several non-antimicrobial resistance outcomes. Information for this review was obtained from the published literature, including reports based on data from national surveillance programs as well as hypothesis-driven research. Most referenced studies focused on the effects of bans on antimicrobial growth promoters (AGPs) and most were conducted in Europe.

Effects of Termination of Use of Antimicrobial Growth Promoters (AGPs)

Usage of antimicrobials

Following the AGP ban in Denmark, therapeutic antimicrobial use in poultry and cattle was unaffected by the ban, however in weaned pigs there were relative increases in therapeutic use of some antimicrobials important for use in humans (tetracycline, penicillins, macrolides, aminoglycosides). Among *Salmonella* Typhimurium (but not *E. coli*) isolates from pigs and domestically acquired infections in humans, there was an increase in resistance to tetracyclines that may have been caused by increased therapeutic tetracycline use in pigs. There was a decrease in macrolide resistance in *Campylobacter* from pigs. Use of cephalosporins and fluoroquinolones was unaffected by the AGP ban. Experience in other countries varied; therapeutic use decreased in Norway, was unaffected in Switzerland, and increased in Sweden and the Netherlands following their AGP bans.

Food safety and human health (other than antimicrobial resistance)

AGP termination in Denmark did not affect the incidence of antimicrobial residues in foods, domestically-acquired human salmonellosis, campylobacteriosis or yersiniosis, nor were there effects on contamination of domestic meat and poultry with *Salmonella* and *Campylobacter.*

From the perspective of global food security, likely decreases in poultry and pork production were estimated to be no more than 2% and average daily protein supply would likely decrease by no more than 0.1 g per person (or 0.2% of total protein intake).

Animal health and welfare

Some countries experienced temporary problems following their AGP bans, mainly diarrhea in weaned pigs and necrotic enteritis in poultry. In Denmark, treatments for post-weaning diarrhea increased from approximately 0.4 to 1.0 treatments per pig-month prior to and after AGP termination in weaners, respectively. Necrotic enteritis diagnoses were made in 25 of 1700 Danish broiler flocks in the year after the ban compared with 1-2 per 1700 flocks annually prior to the ban.

Environment

No evidence was found in Denmark of adverse environmental effects, including total nitrogen and phosphorus output in animal manure.

Animal production

Estimates of the magnitude of AGP adverse effects on production, mainly from experimental studies, vary widely, ranging from approximately 0-15%, however there is evidence that beneficial effects have declined over time, and since the early 2000s range from 0-5%. In Denmark, there were some temporary (two years or less after the ban) production losses detected in weaned pigs, mainly through mortality (0.6% increase), growth rate (2.6% decrease) and feed efficiency (increase of 1-2% in feed units required per weaner produced). No effects on productivity or feed efficiency in finishers were identified. Production effects in Danish broilers were limited to decreased feed efficiency (-2.3%) that was largely offset by savings in the cost of AGPs. In a large U.S. study, removal of AGPs was associated with reduction in livability of 0.14%-0.2%, an average decrease in body weight of 0.03-0.04 lb, and an average increase in feed conversion ratio of 0.012-0.016.

Economic impacts

In Denmark, net costs due to productivity losses from AGP termination were estimated to be 7.75 DKK (1.04 €) per pig produced (1%) and no net cost for poultry. Findings from a general equilibrium model of the Danish economy indicated that AGP termination lowered pig production by about 1.4% per annum and increased poultry production by 0.4% per annum. Impact of AGP termination on the Danish economy was estimated to be a reduction of 0.03% (363 million DKK (48 million €) by 2010 at 1995 prices) in real Gross Domestic Product (GDP). A recent U.S. evaluation estimated that a 1-3% increased cost of production in pigs and broilers would lead to a 1% increase in wholesale prices and drop in output of less than 1%. Another study estimated the potential loss of production and meat value following an AGP ban under two scenarios: 1) effects of AGPs are high (using growth response data from the 1980s), and 2) effects of AGPs are low (using growth response data from the 2000s). They projected that a worldwide ban on AGPs would result in a decrease of global meat production by 1.3% to 3% from its current level (1980s vs. 2000s scenarios). This corresponds to a global loss of between USD 13.5 and USD 44.1 billion in the two scenarios.

Factors that may have mitigated some adverse effects of AGP termination

Some countries that banned AGPs took steps to mitigate possible negative effects of the AGP bans. In Denmark, research was conducted on alternatives to AGPs, and some of these were adopted by the industries.

Investments were made in more efficient pig and poultry production (e.g. enhanced biosecurity, control of disease spread, changes to rations to reduce enteric infection). Additional controls were placed on therapeutic antimicrobial use. Animal production industries continued to improve efficiencies, and adapted well to the lack of AGPs. Other countries also implemented measures; in Sweden and Norway, farmers were provided with extension advice on various aspects of animal management to improve animal health, infection prevention and prudent use of antimicrobials.

Effects of Other Types of Antimicrobial Use Restrictions

In 2010, the Danish Veterinary and Food Administration introduced the “Yellow Card” system to place regulatory restrictions on pig farmers that used twice the average quantity of therapeutic antimicrobials. The impact of the program on slaughter condemnations in pigs at slaughter was evaluated and there were increases in some lesions, but decreases in others.

The Netherlands recently undertook major reductions in antimicrobial consumption in food animals, as well as further restrictions on critically important antimicrobials such as fluoroquinolones and cephalosporins. The Dutch Animal Health Service reported some indications of increased disease problems in pigs, but some of the increases may have been related to feed changes.

Evidence from targeted epidemiological studies indicates that under conditions of good quality veterinary care and animal management, reductions in therapeutic / prophylactic antimicrobial use can be achieved without adverse effects on animal health and production.

Introduction

Restrictions on the use of antimicrobials in food-producing animals generally seek to reduce the presence of antimicrobial-resistant genetic elements and/or antimicrobial resistance in humans and / or animals.

It is also possible that such restrictions may lead to other outcomes, including those related to animal health, animal welfare, overall antimicrobial usage, productivity and cost of food animal production, food safety, zoonotic disease, food security and national economy. There has been considerable attention in the literature and elsewhere on potential negative consequences from bans on antimicrobial growth promoters (AGPs), with considerably less discussion of unintended consequences from restrictions on other types of use (e.g. therapy, prophylaxis).

Methods

Most information for this review was obtained from peer-reviewed and grey literature reports from the handful of national antimicrobial resistance (AMR) and antimicrobial use (AMU) surveillance programs that documented impacts of restrictions on AMU (e.g. Denmark, Norway, Sweden, the Netherlands). This was supplemented with information from published studies of farm-level epidemiological studies. All sources are cited in the text and listed under References. The methods used for searching the literature were informal and non-systematic, but a conscious effort was made to extract and summarize the relevant information in a balanced manner.

Results and Discussion

a) Effects of Termination of Use of Antimicrobial Growth Promoters (AGPs)

The impacts of AGP bans in Sweden and Denmark have for decades been the subject of intense scrutiny and debate; Sweden because it was the first country to take this action in 1986, and Denmark because extensive pre- and post-ban monitoring data were made publicly available and also because it is a leading exporter of pork, with a relatively large, highly evolved food animal industry *(1–8)*. Limited additional data is also available from other European countries. In 2002, WHO convened an expert panel "...to review the potential consequences to human health, animal health and welfare, environmental impact, animal production, and national economy resulting from Denmark's program for termination of the use of antimicrobial growth promoters in food animal production, particularly swine and broiler chicken" *(9)*. The following discussion is organized along the lines of this WHO report, but is supplemented where appropriate with more recent Danish data as well as data from other countries. For some parameters, such as antimicrobial use, available data are limited to European countries that monitored these parameters before and after the bans.

Antimicrobials have been used as growth promoters since the 1950s but their mechanism(s) of action are poorly understood. There is evidence that they have disease prophylaxis properties but may also have other effects on the microbial ecology of the gut that influence feed efficiency and availability of certain nutrients *(9)*.

i) Impact of the termination of antimicrobial growth promoters on usage of antimicrobials

Following the Swedish ban on AGPs in 1986, quantities of therapeutic antimicrobials that were used in animals increased by 21% until 1988, remained stable until 1994, then reduced by 47% by 2003 *(10)*. Most of the temporary increase was in broilers and weaned pigs. Over this period the population of pigs in Sweden declined by 16% and numbers of other species fluctuated, except poultry, which increased. Beginning in 1992, zinc oxide use for prevention of diarrhea in pigs was permitted and became widely used, but was later *(11)*. Norway phased out AGPs in 1995, and there was actually a 39% decrease in therapeutic use from 1995 to 2000, then it remained about the same until 2003. The population of pigs in Norway increased by about 10% from 1995 to 2003 and there were also increases in poultry production *(10)*.

In Denmark, AGP use in cattle, broilers and finisher pigs was terminated in February 1998, and in weaner pigs by the end of 1999. The antimicrobials involved included avoparcin, carbadox, bacitracin, olaquindox, spiramycin, tylosin and virginiamycin. Overall quantities of antimicrobials used in food animals in Denmark declined substantially following the AGP ban (Figure 1). Durations of exposure to antimicrobials were also substantially reduced. Therapeutic use in poultry and cattle was unaffected by the ban, however in pigs (weaned pigs in particular), there were relative increases in therapeutic use of some antimicrobials important for use in humans (tetracycline, penicillins, macrolides, aminoglycosides)*(3,9)*. Among *Salmonella* Typhimurium (but not *E. coli*) isolates from pigs and domestically acquired infections in humans, there was an increase in resistance to tetracyclines that may have been caused by increased therapeutic tetracycline use in pigs. There was a decrease in macrolide resistance in *Campylobacter* from pigs. Use of cephalosporins and fluoroquinolones was unaffected by the AGP ban *(9)*. In the decade following the ban, therapeutic use of antimicrobials in pigs generally correlated with the increasing population of pigs in Denmark (Figure 1).

Switzerland banned AGP use in swine in 1999 and reportedly observed no effect on the use of therapeutic antimicrobials in swine *(12)*. In the Netherlands, termination of AGPs was accompanied by a compensatory increase in therapeutic antimicrobial use, to the extent that there was no overall decrease in antimicrobial use in food animals *(13,14)* (Figure 2). In the United Kingdom, therapeutic antimicrobial use declined following AGP termination, with the exception of macrolides, perhaps to control *Lawsonia intracellularis* infections in pigs. There was no increase in necrotic enteritis in broilers but proliferative haemorrhagic enteropathy in pigs and cholangeohepatitis in broilers increased after the AGP ban *(13)*.

ii) Impact of the termination of antimicrobial growth promoters on food safety and human health (other than antimicrobial resistance)

AGP termination in Denmark did not affect the incidence of antimicrobial residues in foods, domestically-acquired human salmonellosis, campylobacteriosis or yersiniosis, nor were there effects on contamination of domestic meat and poultry with *Salmonella* and *Campylobacter (9)*.

It has been hypothesized that AGPs and prophylactic and / or therapeutic treatment of herds and flocks contributes positively to food safety through reduced risk of carcass contamination with foodborne pathogens *(15)*. Much of the evidence supporting this hypothesis derives from the results of a single observational study that identified a significant association between the presence of air sacculitis in broilers and carcass contamination with *Campylobacter (16)*. It is proposed that lesions and variable carcass weights at slaughter increase the likelihood of intestinal damage and carcass contamination with enteric pathogens *(15)*. Systematic reviews found inconsistency in the effects of in-feed antimicrobials on shedding of *Salmonella* in pigs and poultry and *Escherichia coli* O157:H7 in cattle *(17–20)*.

Collignon and co-workers *(21)* examined the potential impact of a global ban on AGPs on protein undernutrition in developing countries. Likely decreases in poultry and pork production were estimated to be no more than 2% and average daily protein supply would likely decrease by no more than 0.1 g per person (or 0.2% of total protein intake).

iii) Impact of the termination of antimicrobial growth promoters on animal health (morbidity) and welfare

Following termination of AGPs in Sweden in 2006 there were increases in quantities of therapeutic antimicrobials used in pigs as well as problems with increased diarrhea in weaned pigs (estimated 75% of animals), which lasted for about four years, and some problems with necrotic enteritis in poultry. No health problems were encountered in cattle, finisher pigs and turkeys. In-feed antimicrobial use was allowed to continue in broilers to prevent necrotic enteritis (due to *Clostridium perfringens*) for two years until the industry adopted alternative measures *(10,11,13)*.

In Denmark, for approximately two years after the AGP ban there were increased antimicrobial treatments for diarrhea in pigs, mainly in weaners, and to a lesser extent in finishers. In one Danish study, treatments for post-weaning diarrhea increased from approximately 0.4 to 1.0 treatments per pig-month prior to and after AGP termination in weaners, respectively *(9)*. Minor problems with necrotic enteritis were encountered in broilers; diagnoses were made in 25 of 1700 flocks in the year after the ban compared with 1-2 per 1700 flocks annually prior to the ban *(1,9)*. In Finland, termination of the use of growth promoters carbadox and olaquindox in 1999 was not associated with health problems in pigs *(22)*.

iv) Impact of the termination of antimicrobial growth promoters on the environment

The potential environmental effects of AGP termination were evaluated by reviewing the limited available data on heavy metals (e.g. zinc, copper) and soil nutrients (e.g. nitrogen, phosphorus). No evidence was found of adverse environmental effects, including total nitrogen and phosphorus output in animal manure *(9)*. Zinc oxide can reduce the incidence and severity of diarrhea in pigs and was used as an alternative to AGPs for pigs in Sweden and Denmark, but overall usage was not monitored in Denmark immediately before or after the ban. There is some concern about the extent of use of zinc oxide for disease prophylaxis because of resistance co-selection and effects on the environment, but to date extent of use does not appear to exceed environmental guidelines.

v) Impact of the termination of antimicrobial growth promoters on animal production

Numerous studies, mainly experimental, have been conducted to measure the effects of AGPs on various animal production parameters in different species, including average daily gain, days to market, feed conversion ratio, and others *(9,23–25)*. Estimates of the magnitude of effects vary widely, ranging from approximately 0-15%, however there is evidence that beneficial effects have declined over time, and since the early 2000s range from 0-5% *(25)*; (Figures 3a-b). The reasons for this decline are unknown, but it has been suggested that AGPs are most beneficial when animals are housed in conditions of overcrowding and poor hygiene. In recent decades there have been substantial improvements in animal husbandry, especially in poultry and pig production, particularly in housing, nutrition, health management and animal genetics, and these improvements may play a role in reduced efficacy of AGPs *(24)*.

For about two years after AGP termination in Denmark there were some production losses detected in weaned pigs *(9)*, mainly through mortality (0.6% increase), growth rate (2.6% decrease) and feed efficiency (increase of 1-2% in feed units required per weaner produced). No effects on productivity or feed efficiency in finishers were identified. In 2010, further analyses were conducted using additional years of production data and additional parameters, and results showed that over the longer term, AGP termination had little discernible direct effect on several production indices (e.g. mean number of pigs farrowed per sow per year, mortality rate average daily gain, number of feed units, and percentage of dead or condemned finishing pigs) *(2)*; (Figure 4a-c).

Production effects in Danish broilers were limited to decreased feed efficiency (-2.3%) that was largely offset by savings in the cost of AGPs. Kilogram broilers produced per square meter and percent dead broilers in total were not affected by the termination of AGPs *(9,26)*.

An example of poultry industry research in this area is a set of large-scale field studies (7 million broilers over 3 years) conducted by a major poultry producer (Perdue) in two regions of the United States *(27)*. Flocks were allocated to either rations containing the organoarsenic coccidiostat roxarsone plus AGPs (bacitracin, flavomycin and virginiamycin), or rations containing roxarsone without AGPs. Depending on the region, removal of AGPs was associated with reduction in livability of 0.14%-0.2%, an average decrease in body weight of 0.03-0.04 lb, and an average increase in feed conversion ratio of 0.012-0.016. Skin color scores and field condemnations were not significantly negatively impacted but there was less uniformity in body weights without AGPs.

vi) Economic impacts of the termination of antimicrobial growth promoters

There are many factors that may affect the economic impact of AGP termination in various species of food animals (Table 1).

Analyses of the economic impacts of AGP termination in Denmark were conducted for the WHO review *(9)*. Net costs due to productivity losses from AGP termination in pig and poultry production were estimated to be 7.75 DKK (1.04 €) per pig produced (1%) and no net cost for poultry. Losses in pigs were attributed to excess mortality and feeding days, and increased medication and workload; some potential costs (e.g. farm infrastructure) were not measured. Findings from a general equilibrium model of the Danish economy indicated that AGP termination lowered pig production by about 1.4% per annum and increased poultry production by 0.4% per annum. Poultry production was considered to indirectly benefit from lower pig production. Impact of AGP termination on the Danish economy was estimated to be a reduction of 0.03% (363 million DKK (48 million €) by 2010 at 1995 prices) in real Gross Domestic Product (GDP). Not included in this analysis were any benefits from increased consumer confidence, increased demand following AGP termination, and economic value of any human health benefits.

Graham et al *(23)* used data from the large field studies of AGPs in broilers in the U.S. *(27)* to estimate the economic effect of removing AGPs from poultry rations. They considered the cost of feed with AGPs, feed conversion ratio, and change in value of a flock as a function of differences in weight gain, mortality, and condemnation. The net effect of using AGPs was a lost value of $0.0093 per chicken (about 0.45% of total cost), but this did not offset the cost of the AGPs.

Various researchers estimated the potential economic effect of an AGP ban on the U.S. swine industry (reviewed in *(25)*). These estimates have ranged widely, e.g. $0.59/pig and 9% decrease in net profits, $2.33/pig and 2% increase in production costs, and $4.50/pig in the first year and a 4.5% increase in overall production costs.

Sneeringer and co-workers *(28)* estimated effects of termination of the use antimicrobials for production purposes (growth promotion) on production, prices, and total revenue. They estimated that a 1-3% increased cost of production in pigs and broilers would lead to a 1% increase in wholesale prices and drop in output of less than 1%. They reported little research on the productivity effects of AGPs in cattle. The authors reviewed U.S. data and observed that AGP use is not universal and declining, perhaps because of reduced efficacy as shown in research since 2000. They concluded that U.S. producers, like those in Europe, would likely adopt alternative practices in place of AGPs.

Laxminarayan and co-workers *(24)* recently estimated the potential loss of production and meat value following an AGP ban under two scenarios: 1) effects of AGPs are high (using growth response data from the 1980s), and 2) effects of AGPs are low (using growth response data from the 2000s). They projected that a worldwide ban on AGPs would result in a decrease of global meat production by 1.3% to 3% from its current level (1980s vs. 2000s scenarios). This corresponds to a global loss of between USD 13.5 and USD 44.1 billion in the two scenarios.

vii) Factors that may have mitigated some adverse effects of AGP termination

As pointed out by the WHO Expert Panel that reviewed the extensive Danish monitoring data related to bans on antimicrobial growth promoter use, national level data tend to reveal overall effects on national populations rather than highlight specific effects on individual producers *(9)*. Some producers may have experienced more difficulties, either because of the nature of their facilities, management, and exposure to disease agents, and perhaps also because of the naturally occurring biological range of variability. By the same token, some other producers would have experienced no adverse effects.

Denmark took several steps to mitigate possible negative effects of the AGP bans but their impacts were not specifically measured. Extensive research was conducted on alternatives to AGPs, e.g. probiotics, prebiotics, feed changes, vaccines. Some of these were adopted by the industries (e.g. increased use of whole wheat and feeding enzymes in poultry rations). Denmark also invested in more efficient pig and poultry production (e.g. enhanced biosecurity, control of disease spread, changes to rations to reduce enteric infection) *(1,9,29)*. Some changes were adopted for multiple reasons, only one of which may have been AGP termination *(9)*. These changes may have mitigated some effects of AGP termination on production parameters and disease incidence, but to an unknown extent. Other countries also implemented mitigating measures; in Sweden and Norway, farmers were provided with extension advice on various aspects of animal management to improve animal health, infection prevention and prudent use of antimicrobials *(10,13)*.

Additional measures were taken in Denmark to limit increases in prophylactic and therapeutic antimicrobial use, including removal of veterinary profit from sales of antimicrobials, introduction of a "cascade rule" to prevent overuse of cheap compounds such as tetracycline, restrictions on the use of generalized flock treatments, limitations on prescribing antimicrobials for longer than 5 days, and prohibition of pharmacies and industry from offering economic incentives to promote sales *(29)*.

The Danish broiler industry expected problems with necrotic enteritis following the AGP ban so a compensation fund was established to offset losses to producers. In the first year following the ban necrotic enteritis was diagnosed in 25 of 1700 flocks compared to 1-2 per 1700 flocks annually prior to the ban. Only 15% of the compensation fund was spent, so the industry felt confident that the problem was not under-diagnosed. Ionophore antimicrobials continued to be used to prevent coccidiosis (enteric disease caused by a protozoan parasite) and this may also have prevented some outbreaks of necrotic enteritis *(9)*.

b) Effects of Other Types of Antimicrobial Use Restriction

i) Danish "Yellow Card" Program

In 2010, the Danish Veterinary and Food Administration introduced the "Yellow Card" system to place regulatory restrictions on pig farmers that used twice the average quantity of antimicrobials. This resulted in a substantial reduction in antimicrobial use in Danish pigs *(1,29)*. Alban et al *(30)* evaluated the impact of the program on vaccine usage in the swine industry and specific lesions detected in pigs at slaughter for one year in 2010 and 2011, before and after introduction of the program, respectively. No differences were detected in rates of osteomyelitis, pleuritis, and chronic arthritis before and after program introduction. The prevalence of chronic peritonitis, umbilical hernia and chronic enteritis were statistically higher in 2011 compared to 2010, but lower for tail bite infection, chronic pericarditis, and chronic pneumonia. In 2011, there was higher use of vaccines against respiratory diseases. The costs associated with implementation of the Yellow Card system were estimated to be approximately € 1 million per annum *(6,30)*.

ii) Dutch Program to Reduce Non-AGP Antimicrobial Use

In the Netherlands following the AGP ban in 2006 there was no overall decrease in antimicrobial consumption in animals *(14)*. Desiring reduction in quantities used for disease prophylaxis and therapy, the Dutch government set mandatory consumption reduction targets of (compared to 2009 levels) 20% in 2011, 50% in 2013 and 70% in 2015. At the same time, certain other restrictions were applied, including no use of new antimicrobials (e.g. carbapenems) in animals, use of fluoroquinolones and cephalosporins permitted only with evidence that other antimicrobials would be ineffective, and colistin, betalactams and aminoglycosides were made second choice antibiotics. In a report from the European Medicines agency *(5)* it was stated that shortly after initiation of the reduction program the Dutch Animal Health Service GD reported some indications of increased disease problems in pigs, including diarrhea and more *E. coli* infections and an increase in the number of dead animals submitted for pathological investigation. Some of the increases may have been related to feed changes *(5)*. It was suggested that there has not been sufficient time to fully assess impacts of the reduction program.

In addition, in 2009 the Dutch poultry industry voluntarily discontinued use of fluoroquinolones and 3rd generation cephalosporins, and in 2013 the swine industry voluntarily discontinued use of 3rd generation cephalosporins. No adverse effects from these voluntary bans were reported *(5)*.

de Jong et al *(31)* investigated the relationship between reduction in antimicrobial use and mortality and hock burn in broilers. They did not find a clear relationship and there were no difference in mortality and hock burn in treated compared to untreated flocks. A survey of farmers indicated a belief that the program resulted in an increase in the number of rejections of broilers at slaughter and the number of chickens culled on the farm.

iii) Organic production

Several studies have shown that broilers reared under organic production conditions have higher prevalence of *Campylobacter* than those reared under conventional production *(6).* It has been pointed out that at least in Denmark, this difference could reflect that organic flocks are grown outdoors and have higher age, both known risk factors for *Campylobacter* infection in poultry *(32).* Young et al *(33)* conducted a systematic review of published literature on the prevalence of bacterial enteropathogens and potentially zoonotic bacteria in organic and conventional poultry, swine and beef production. They found that the prevalence of *Campylobacter* was higher in organic broiler chickens at slaughter, but there was no difference in prevalence in retail chicken. They found limited or inconsistent results on the prevalence of zoonotic and potentially zoonotic bacteria in other food-animal species *(33).*

iv) Other Studies

Berge et al. *(34)* studied antimicrobial use in pre-weaned dairy calves on a single farm. In groups of calves given antimicrobial treatment only when the animals were clinically sick, there was significantly less diarrhea compared to groups where animals were administered prophylactic antimicrobials, and there were savings of around 10 US$ per calf in the targeted treatment groups.

Dorado-García et al *(35)* conducted a study of interventions to curb livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) using 51 veal calf farms in the Netherlands. There was an overall decrease in antimicrobial use (daily dosages per animal per cycle (DDDA/C)) over the course of the study and this was not associated with technical parameters studied (mortality, carcass weight, veterinary costs, duration of production cycle).

Rojo-Gimeno et al *(36)* examined costs associated with reducing antimicrobial use on Belgian pig farms while improving health management strategies. They estimated that the costs of new biosecurity measures and vaccinations did not exceed savings from reduced use of antimicrobials. No negative effects on production technical parameters were observed and mortality in finishers was significantly reduced by −1.1%. After a substantial reduction in antimicrobial usage and implementing improved management strategies, the difference of the enterprise profit increased by +D 2.67/finisher pig/year.

Postma et al *(37)* studied reduction in antimicrobial use and impact on health and production parameters on 61 Flemish pig farms. Along with efforts to improve prudent antimicrobial use were interventions to optimize herd management, biosecurity status, vaccination strategy, and anthelmintic therapy. Antimicrobial use was reduced by 52% from birth to slaughter and 32% in breeding animals, including important reductions in the use of critically important antimicrobials. Improvements in production (including number of weaned piglets per sow per year, daily weight gain and mortality in the finisher period) were observed. The authors concluded that is possible to reduce antimicrobial use without loss of production if the herd veterinarian and producer work together.

Antimicrobial resistance may have adverse impacts on animal health, just as it does on human health. Resistance to antimicrobials important to animal health has been documented in several important bacterial pathogens of food animals, including *Brachyspira hyodysenteriae Escherichia coli*, *Histophilus somni*, *Pasteurella multocida*, *Mannheimia haemolytica* and *Staphylococcus aureus (38,39)*. However, to our knowledge, there have been no attempts to determine whether restrictions on antimicrobial use in animals have resulted in decreased antimicrobial resistance among bacteria of purely animal health importance (i.e. other than *Salmonella*, *Campyloacter* and *E. coli*).

Conclusions

Overall, the adverse consequences of AGP bans and other restrictions described in the literature appear to be limited and temporary.

Based on the experiences in Europe with terminating AGPs, other countries considering similar action should put in place measures to minimize disease in vulnerable classes of animals, especially weaner pigs. Effects of AGP bans in other countries may vary, depending on the antimicrobial classes used as AGPs. Particular care is needed to avoid compensatory increases in antimicrobial use for disease prophylactic or therapeutic purposes, especially antimicrobials important for therapy in either humans or animals.

The European experience suggests that prescription-only availability of antimicrobials is not usually sufficient on its own to prevent post-AGP ban compensatory increases in antimicrobial use for therapy or disease prophylaxis. Additional measures are needed, e.g. antimicrobial use surveillance linked to remedial action on excessive use, mandatory antimicrobial use reduction targets, and improvements in animal health management.

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Table 1. Potential Economic Effects of AGP Restrictions at Animal, Farm, and Market Levels (reprinted from *(25)*).

Potential Economic Effects of Withdrawing AGPs	
Potential Costs	**Potential Benefits**
Potential Animal-Level Effects	
Decreased growth rate, decreased feed efficiency	---
Short term higher mortality rate (especially of young animals), increased morbidity	Long term improvement in health status of animals after investing in biosecurity measures.
	Potential preservation of antimicrobial efficiency to treat animals.
Fewer animals born per litter	---
Increased variability of product	---
Potential Farm-Level Effects	
Increased time to market and decreased stocking densities	---
Increased input costs: feed (non AGP), young animals purchased	Decreased input costs: saving in AGP cost
Cost of more biosecurity measures and adjustments in housing to compensate for AGP termination	Long term improvement in health status of animals. Decrease in transmission of all diseases, including diseases which are not prevented by antimicrobials (e.g. viral diseases, respiratory tracts infections).
Increased veterinary costs (more treatment of disease)	Decreased veterinary costs (less disease outbreak after having invested in biosecurity measures)
Higher labor costs if alternatives to AGP are more labor-intensive	---
Increased variability of product	---
Potential Market-Level Effects	
Supply side: less output for each level of input, increase in wholesale and retail price of meat, variation in producers revenues (increase or decrease)	Supply side: Potential increase in producers revenues (increase in wholesale price of meat)
---	Demand side: increased consumer confidence and demand for product; increased access to export markets that previously rejected U.S. products because of AGP use

Figure 1. Antimicrobial consumption and millions of heads of pigs produced in Denmark from 1994 to 2013. Black line indicates number of heads. Bars indicate total antimicrobial consumption adjusted to mg kg−1 of pork produced (grey indicates use as antimicrobial growth promoter (AGP), white used for treatment). Also depicted are important events over time, including: no sales profit in 1995 reducing the use of antimicrobials for treatment, ban of the AGP avoparcin in 1995, ban of the AGP virginiamycin and voluntary stop for all AGP use in 1998, complete stop for all AGP use at the end of 1999 and implementation of the yellow card scheme in 2010. (reprinted from *(1)*).

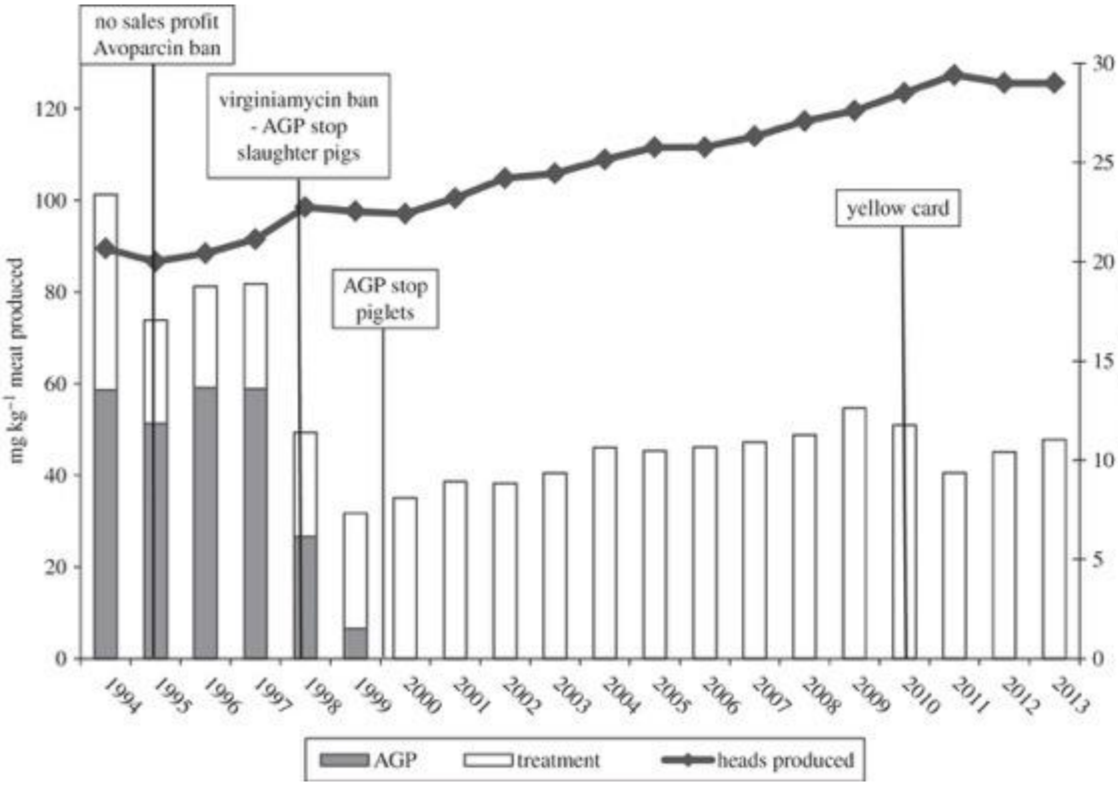


Figure 2. (courtesy Jaap Wagenaar).

Effect of the ban of AGPs on Dutch AMU in animals

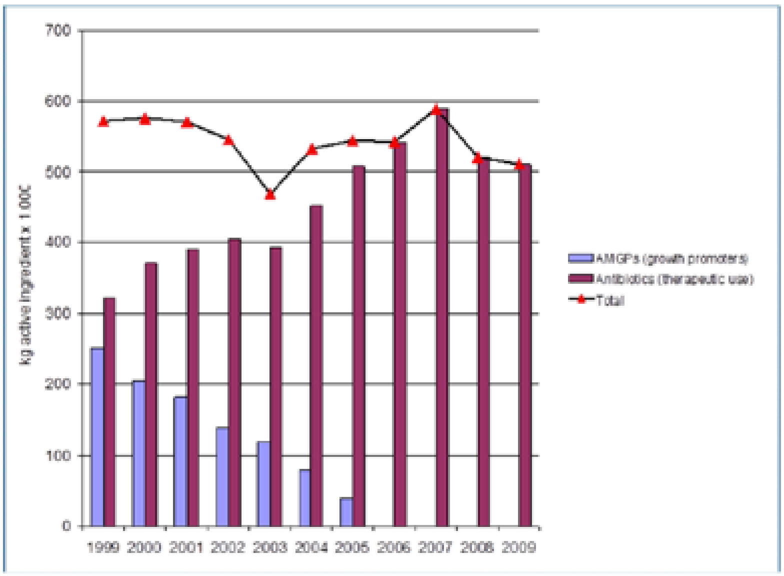


Figure 3a. Percentage Improvement in Average Daily Growth of Pigs Fed Antibiotics over Time (reprinted from *(25)*).

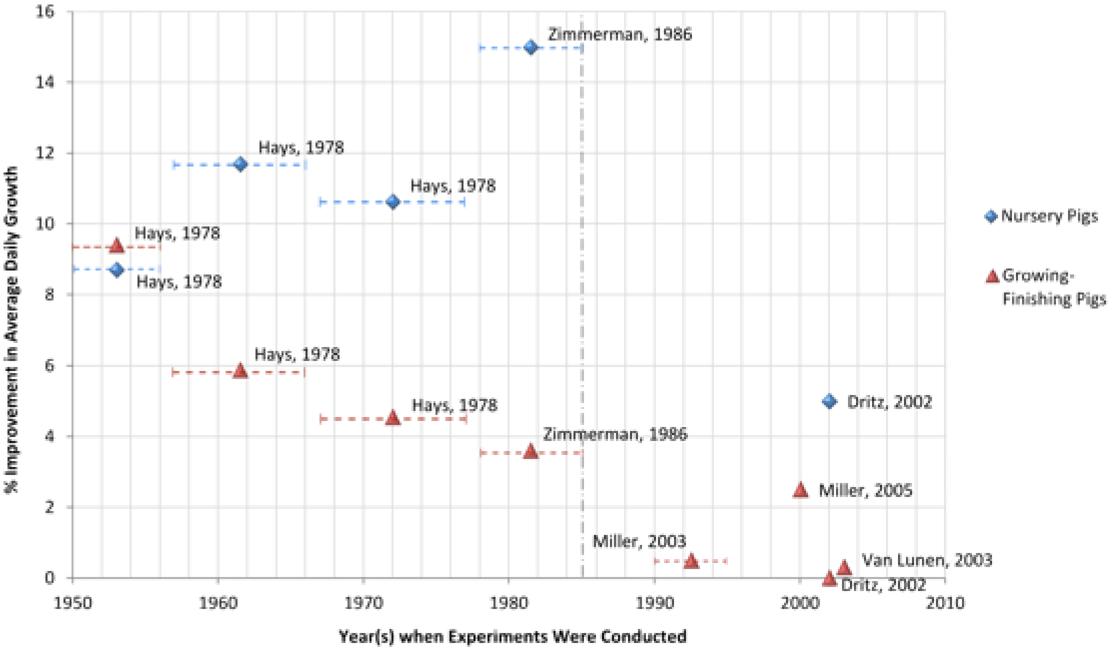
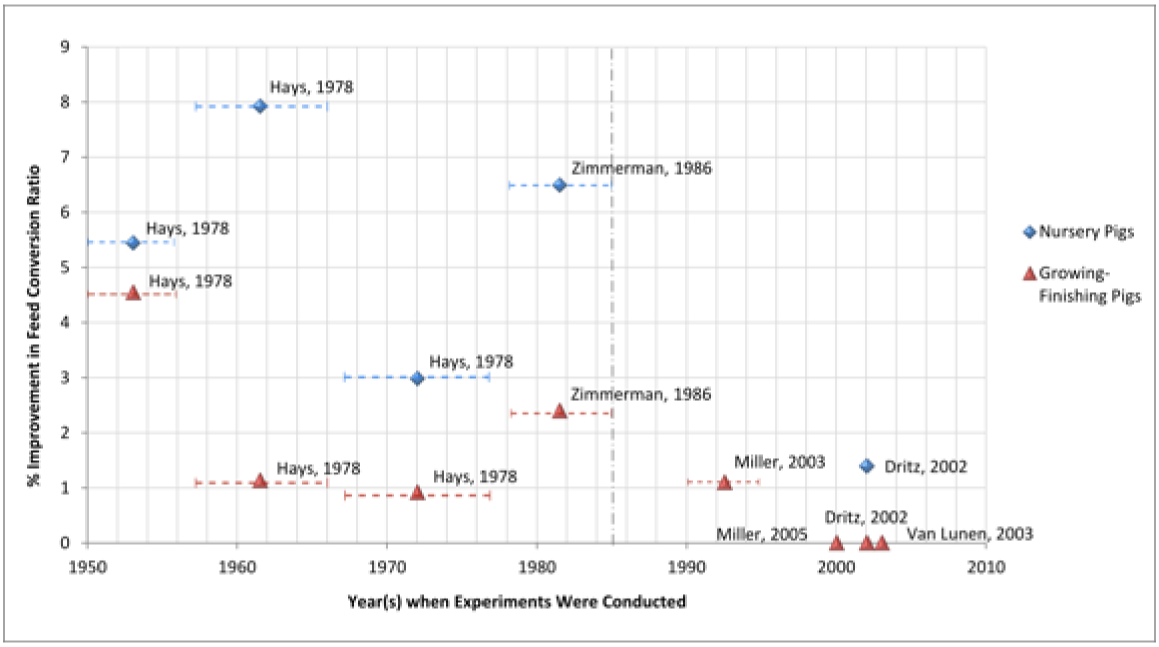
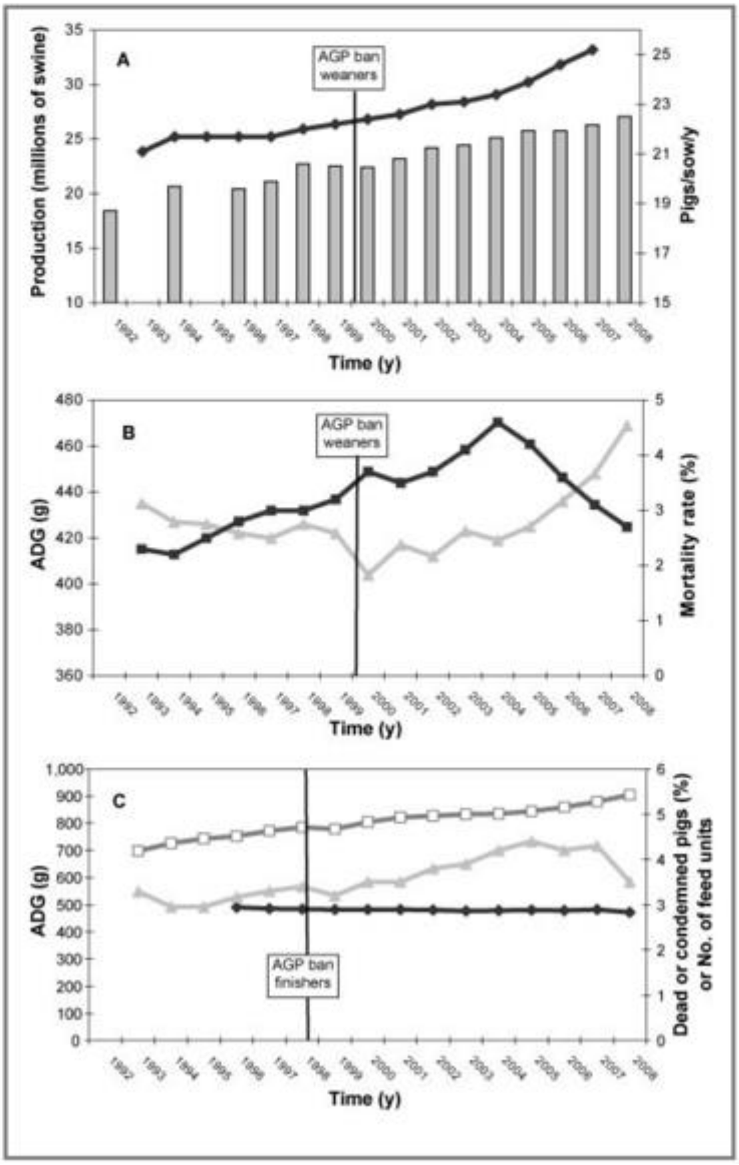


Figure 3b. Percentage Improvement in Feed Conversion Ratio of Pigs Fed Antibiotics over Time (reprinted from *(25)*).



Note: The x-axis refers to the year when the experiments were conducted. Hays, 1978 and Zimmerman, 1986 are reviews of studies conducted over a given time period. The horizontal lines represents the period during which the experiments were conducted. The vertical dashed line separates early vs recent studies.

Figure 4. Data of production characteristics for total production of pigs (millions of swine; gray bars) and mean number of pigs farrowed per sow per year (black diamonds; A), ADG (gray triangles) and mortality rate (black squares) in weaning pigs (B), and ADG (white squares), number of feed units (black diamonds) and the percentage of dead or condemned finishing pigs (gray triangles; C) raised in the Danish swine production system from August 15, 1991, through November 14, 2007. Data for total pig production were collected during the calendar year from January 1 through December 31; all other production values were collected from August 15 through November 14 of the following year and reported as the year in which the data collection period terminated. The ban on AGP use (vertical line) was instituted on April 1, 1998, and January 1, 2000, for finishing and weaning pigs, respectively. Weaning and finishing pigs weighed < 35 kg and > 35 kg, respectively. (reprinted from *(2)*).



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