Use of Quinolones in Food Animals and Potential Impact on Human Health

Report and Proceedings of a WHO Meeting

Geneva, Switzerland
2-5 June 1998

Division of Emerging and other Communicable Diseases Surveillance and Control
WORLD HEALTH ORGANIZATION
CONTENTS

I. INTRODUCTION .................................................................................................................. 1

II. USE OF QUINOLONES IN HUMANS AND EMERGING RESISTANCE PROBLEMS ........................................ 3

III. LINKS BETWEEN QUINOLONE USE IN FOOD ANIMALS AND OBSERVED INCREASES IN QUINOLONE-RESISTANT FOOD-BORNE PATHOGENS AND HUMAN TREATMENT PROBLEMS ........................................ 5
    General statements ........................................................................................................... 5
    Evidence in relation to Campylobacter ............................................................................. 5
    Nature of resistance ......................................................................................................... 5
    Campylobacter in food animals ....................................................................................... 5
    Impact on human health .................................................................................................. 6
    Evidence in relation to non-typhoidal Salmonella .......................................................... 6
    Nature of resistance ......................................................................................................... 6
    Salmonella in food animals ............................................................................................. 6
    Impact on human health .................................................................................................. 7

IV. QUINOLONE USE IN ANIMALS AND RESISTANCE IN ANIMAL BACTERIA .................................................. 9
    Quinolone use in animals ............................................................................................... 9
    Licensing and post-licensing procedures and requirements for quinolones in various parts of the world .......................................................... 12
    Quinolone resistance patterns in animal pathogens and commensals .......................... 12

V. RECOMMENDATIONS ....................................................................................................... 14
    Research needs ............................................................................................................... 14
    Data gathering needs ..................................................................................................... 15
    Monitoring of antimicrobial resistance ........................................................................ 15
    Usage of quinolones in food animals ............................................................................ 16
    Prudent use of antimicrobials in livestock ..................................................................... 16
    Aquaculture .................................................................................................................. 17

LIST OF PARTICIPANTS AND OBSERVERS ...................................................................... 19

AGENDA AND TIMETABLE ................................................................................................. 23
CONTENTS cont.

WORKING PAPERS ........................................................................................................ 27

1. Mechanisms of Quinolone Resistance ................................................................. 27

2. Susceptibility Testing of Quinolones and Links Between
   In Vitro and In Vivo Resistance ........................................................................... 35

3. Review of the Clinical Use of Quinolones in Human Medicine:
   Western Hemisphere ............................................................................................. 49

4. Review of the Clinical Use of Quinolones in Human Medicine:
   Eastern Hemisphere ............................................................................................... 59

5. Quinolone Resistance in Community-Acquired
   Pathogens in South Africa ................................................................................... 69

6. Resistance to Quinolones in Zoonotic Bacteria in China .................................. 77

7. Review of Quinolone Resistance in Human Pathogens ..................................... 81

8. Initial Attempts to Assess the Environmental Fate of the Veterinary
   Fluoroquinolone Enrofloxacin by In Vitro Degradation Studies
   Employing Basidiomycetous Fungi Isolated from Wood, Agricultural
   Soils, and Cattle Dung ......................................................................................... 89

9. Overview of Quinolone Usage for Food-producing Animals ............................ 97

10. The Authorisation of Antimicrobials in the EU for
    Veterinary Use ..................................................................................................... 103

11. Quinolone Licensing for Use in Food Animals and Related
    Problems Facing Regulators in the USA ........................................................... 115

12. Overview of Fluoroquinolone Use in US Poultry Production .......................... 119

13. Quinolone Usage in Poultry Medication in Europe ......................................... 123

14. Quinolone Usage and Resistant Bacteria in Chickens in Japan ....................... 133

15. Examples of Quinolone Usage in Pig Production in
    Latin America ...................................................................................................... 139

16. Review of Quinolone Usage in Pig Production .................................................. 145

17. Review of the Use of Quinolones in Food Animals ......................................... 153

II Use of Quinolones in Food Animals and Potential Impact on Human Health
18. Examples of In Vitro Quinolone Resistance Prevalence/Trends in Human and Animal Isolates of Foodborne Salmonella in Australia ................................................................. 163

19. Examples of In Vitro Quinolone Resistance Prevalence/Trends in Foodborne Salmonella and Campylobacter in Denmark ................. 169

20. Examples of In Vitro Quinolone Resistance Prevalence/Trends in Foodborne Salmonella and other enterics in Germany ...................... 175

21. Quinolone Resistance in E. coli and Salmonella spp. Isolated in Japan ......................................................................................... 179

22. Examples of In Vitro Quinolone Resistance Prevalence Trends in Humans and Animal Isolates of Food-borne Salmonella and Campylobacter in the Netherlands ........................................ 181

23. Quinolone Resistance in Campylobacter and Salmonella Strains Isolated in Swedish Patients with Diarrhoea ................................. 187

24. Quinolone-Resistant Campylobacter in the United Kingdom .......... 189

25. Resistance to Fluoroquinolone Antibiotics in Salmonellas from Humans in England and Wales — the Current Situation .................. 199


27. The Origins and Consequences of Antimicrobial-Resistant Nontyphoidal Salmonella: Implications for Use of Fluoroquinolones in Food Animals .................................................. 205

28. Prevalence/Trends of Quinolone Resistance in Salmonella Isolates from Animals in the USA ....................................................... 221

29. Overview on Human Salmonella Infections ...................................... 229

30. Overview of Campylobacteriosis Treatment Problems .................. 233

31. Quinolone Use in Agriculture and Medical Problems — How Reliable is the Data? ................................................................. 245

32. Risk Assessment: Data Needs and Methods .................................. 251
I. Introduction

A variety of antimicrobial types are used in livestock production. Their use inevitably leads to the selection of resistant forms of bacteria in the ecosystem of use. This selection will occur with all uses in livestock production including treatment, prophylaxis and growth promotion.

Priority medical problems arising from the use of antimicrobials in livestock production have been summarized recently. Use of quinolones in livestock has been identified as a particular area of concern because of the significance of this group of antimicrobials for the treatment of a broad range of infections in humans including gastrointestinal infections caused by zoonotic bacteria transmitted to humans via the food chain.

Currently, several quinolones are available for treatment of animals, poultry and fish in many countries in the world. Available data indicate that they are also used for disease prevention in some regions. Quinolone production and usage is estimated to be about 50 tonnes for proprietary products (mainly USA, European Union, Japan, South Korea) and, because of their lower prices, about 70 tonnes for generic quinolones. However, available usage data, particularly for non-proprietary quinolones, are known to be grossly incomplete. For instance, data from China estimate annual quinolone consumption in animals in China alone to be in the range of 470 tonnes (annual consumption in human medicine in China: about 1,350 tonnes).

Considerable variation in the usage and in the regulatory processes for drugs of different countries is recognized to occur. Licensing requirements range from highly controlled to minimal scrutiny and in some cases the products are available without a review process by a regulatory authority. In some countries there is no legal framework for prescription of veterinary pharmaceuticals.

An increase in antimicrobial resistance in zoonotic bacteria isolated from animals, food and humans (e.g., Salmonella and Campylobacter) has already been reported, but the scope of the problems still needs to be identified and the links between quinolone use in animals and the occurrence of problems in infectious disease treatment in humans elucidated.

Against this backdrop, WHO organised a consultation to address some of the above mentioned concerns in June 1998. The objectives of this meeting were to:

- Identify known and potential links between the emergence and spread of quinolone resistance from food-borne and other possible zoonotic bacteria, and human zoonotic infection.
- Review the conditions and extent of use of the various quinolones in humans and in food animals.
- Identify and make specific recommendations on areas of applied research or data gathering that would assist in risk assessment.
The meeting gathered 59 experts from a wide range of disciplines including clinical medicine, infectious disease epidemiology and control, microbiology, veterinary medicine, animal production, licensing and registration of antimicrobials, research and development and sale of antimicrobials. In addition, representatives from numerous governmental and non-governmental organizations and observers attended the consultation.

Thirty-two presentations were delivered by participants of this meeting. Of these papers, 30 were distributed to an e-mail discussion group for comments over a 4-week period prior to the meeting in Geneva. The group included over 450 subscribers from at least 44 countries on all continents. Comments from the group were supplied to meeting participants along with the working papers, at the beginning of the meeting.

Presentations and discussions on the first two days of the meeting reviewed:

- use of quinolones and resistance in humans
- production, licensing and use of quinolones for food animals
- links between quinolone resistance in food animal bacteria and human disease.

One paper provided an overview on the possibilities and conditions of risk assessment in the context of containment of antimicrobial resistance.

Subsequently, two working groups drafted reports which were discussed and adopted during the final plenary session:

- Links between quinolone use in food animals and observed increases in quinolone-resistant food-borne pathogens and human treatment problems
- Quinolone use in animals and resistance in animal bacteria.

This report presents the findings, conclusions and recommendations of this meeting.
II. Use of quinolones in humans and emerging resistance problems

Since the development of newer quinolones and their release in the mid-1980s, there has been extensive clinical use of these agents in human medicine, and four new agents have been released in the past two years. Various quinolones have been approved and used extensively for treatment of a broad range of clinical infections, including those of the genitourinary, gastrointestinal, and respiratory tracts as well as infections of bone, joints, and skin. In the context of increasing resistance of gram-negative bacteria to other classes of antimicrobials, these agents have provided valuable alternative therapies and often been used in place of more toxic aminoglycosides. The most recently released members of the class, which have increased potency against gram-positive and in some cases anaerobic bacteria have also expanded the range of applications particularly to include intra-abdominal infections caused by mixtures of anaerobic and gram-negative bacteria and infections caused by Streptococcus pneumoniae, which has become increasingly resistant to other antimicrobials normally used for treatment of these infections.

With the use of quinolones in human medicine in the past decade, there has also been recognition that this use may select for resistance in human pathogens. Although many pathogens have remained susceptible (e.g., community strains of Enterobacteriaceae) with little increasing resistance in the past decade in many developed countries, there are several circumstances in which resistance has limited therapeutic use. Resistance was first recognized and has had the largest effect in Staphylococcus aureus, especially methicillin-resistant strains, and Pseudomonas aeruginosa, an occurrence which may in part reflect two factors. One factor is the finding that single mutations occurring on the chromosome of members of these species may be sufficient to cause clinically relevant levels of resistance, and the second factor is that infections with these organisms often occur in hospital settings in which nosocomial spread may occur and amplify the prevalence of resistance. Resistance, however, has also occurred in pathogens whose susceptibility is sufficient to require more than a single chromosomal mutation to develop levels of clinical resistance. One particularly important example is resistance in Escherichia coli isolated from blood cultures from neutropenic patients given quinolones for prophylaxis in several medical centers. Epidemiologic and microbiologic studies of these patients have clearly identified quinolone use as the principal risk factor, the occurrence of selection in distinct strains that are part of endogenous fecal flora (rather than nosocomial spread), and the presence of multiple mutations contributing to resistance. These data imply that even for highly susceptible organisms, in settings of intense selection pressure, highly resistant isolates may emerge when there is a large reservoir of organisms such as in the gastrointestinal tract that allows persistence of first-step resistant mutants from which second and later step mutants may be selected. Resistance emerging in urinary isolates of E. coli in Europe and resistance in Neisseria gonorrhoeae have also been associated with human fluoroquinolone use. There is concern that expanded use of quinolones for treatment of respiratory tract infections may pose a risk for development of resistance in S. pneumoniae, but data are currently limited.
For zoonotic infections, fluoroquinolones have often been used for treatment of human infections with *Campylobacter jejuni* and species of non-typhoidal *Salmonella*. For empiric treatment of bacterial gastroenteritis, which is most often caused by *C. jejuni, Salmonella, Shigella, or E. coli*, the fluoroquinolones are one of the few classes of agents with activity against the full range of pathogens, and thus are commonly used. Patients with bacteraemic *Salmonella* infection have also been treated with quinolones. Resistance has been documented to emerge during therapy in small numbers of cases of *Campylobacter* and *Salmonella* with a few clinical failures, but the magnitude of this problem is uncertain. In treatment of enteric fever due to *Salmonella typhi*, a non-zoonotic member of the genus, it has recently been documented that reduced susceptibility to fluoroquinolones without complete resistance (as defined by established breakpoints used in clinical laboratories) is associated with slower rates of response to quinolone therapy, raising concerns that reduced susceptibility without complete resistance in other species of *Salmonella* may affect the outcomes of quinolone therapy.
III. Links between quinolone use in food animals, observed increases in quinolone-resistant food-borne pathogens and human treatment problems

General statements

Quinolones are synthetic antibiotics that act by inhibiting DNA gyrase and topoisomerase IV in susceptible bacteria. The original quinolones have modest activity against Enterobacteriaceae and some other facultative gram-negative bacteria only. Fluorinated quinolones, called fluoroquinolones, were developed from the original quinolones and extended the spectrum of activity to include *Pseudomonas aeruginosa* and some gram-positive bacteria, as well as having substantially increased activity against other gram-negative bacteria.

Bacteria resistant to the original class of quinolones (e.g., nalidixic acid, oxolinic acid) may have reduced susceptibility or resistance to fluoroquinolones. Bacteria resistant to one fluoroquinolone are generally cross-resistant to other fluoroquinolones. This cross resistance includes fluoroquinolones used in animals and those available for human use.

Exposure to lower concentrations of quinolones increases the chance for selection of resistance. This circumstance could be encountered in certain dosing situations, with poor compliance or with poor quality products where inadequate doses could be taken by some individuals. There is controversy about the relative risks of inadequate dosing in an animal population by different routes of administration, particularly the relative risks of dosing via water and feed in comparison to other routes.

Evidence in relation to Campylobacter

*Nature of resistance*

*C. jejuni* and *C. coli* are naturally less susceptible to fluoroquinolones than many other gram-negative bacteria. In these species, single mutations can lead to resistance that exceeds commonly accepted breakpoints. In addition to quinolone resistance, co-resistance with other antibiotics such as macrolides has been noted in Spain and Thailand.

*Campylobacter in food animals*

*C. jejuni* is a frequent commensal in poultry and cattle, and *C. coli* is a frequent commensal in swine and poultry. There is a temporal association between the introduction of fluoroquinolones for use in poultry and a substantial rise in the prevalence of quinolone-resistant *C. jejuni* isolated in live poultry, poultry meat and from infected humans. Moreover, prior to any use in poultry, no resistant strains were reported in individuals with no previous exposure to quinolones.
Impact on human health

*Campylobacter* species are the commonest cause of bacterial gastroenteritis in developed countries. Sporadic cases of campylobacteriosis, which comprise the largest number of reported cases, are predominantly associated with consumption of contaminated food, particularly poultry, in most developed countries.

The majority of gastrointestinal *Campylobacter* infections do not require antibiotic treatment and are self-limiting. Where treatment is felt to be required, erythromycin is usually recommended. However, fluoroquinolones are often used for empiric treatment because they will cover other bacterial pathogens pending laboratory results. They also are better tolerated than erythromycin.

In studies in several countries, there is an association with overseas travel to areas of high prevalence of fluoroquinolone-resistant campylobacter and acquisition of such strains. However, the majority of fluoroquinolone-resistant campylobacter are domestically acquired. In the past, some studies using molecular markers have shown a link between human and animal isolates of susceptible campylobacter. Recently one US study has confirmed similar links for fluoroquinolone-resistant strains.

The effect of fluoroquinolone resistance in campylobacter on the clinical outcome of treatment with a fluoroquinolone is not clear. In immunocompromised patients there have been reports of failures associated with resistance emerging during treatment. However, there are few published data on immunocompetent individuals. There are conflicting data on whether resistant campylobacter have caused more severe disease.

Evidence in relation to non-typhoidal Salmonella

Nature of resistance

Salmonellae are normally highly susceptible to fluoroquinolones. They require two or more mutations to become resistant at levels above the most widely used clinical breakpoints (e.g., ciprofloxacin 1-4 mg/L).

*Salmonella in food animals*

Salmonellae have a wide host range that includes humans and animals, both as a coloniser and a cause of disease. There is a temporal association between the introduction of fluoroquinolones in food-producing animals and the emergence of reduced susceptibility to ciprofloxacin in *S. typhimurium* DT104, already resistant to five antibiotics, in the UK. There are also indications of reduced susceptibility to ciprofloxacin in human isolates of a variety of zoonotic salmonellae following introduction into food-producing animals of fluoroquinolones in the UK, USA and Denmark.
Subsequent to the introduction in 1988 of fluoroquinolones for food animal use in Germany, the emergence of fluoroquinolone-resistant variants of the multi-resistant *S. typhimurium* clone DT204c was observed. Resistance reached a prevalence of 50% in isolates from calves in a defined area of the country. Subsequently the prevalence of these resistant strains has diminished, but data associating this change in prevalence with changes in fluoroquinolone usage in animals are unavailable.

There is uncertainty about the relative contribution of direct selective pressure versus the spread of resistant strains in the presence or absence of quinolone use to the emergence and dissemination of quinolone-resistant Salmonella.

Variation is reported in the rate of emergence of animal strains with reduced susceptibility to fluoroquinolones in different countries after the introduction of fluoroquinolones for use in food animals. Lack of data on usage of quinolones in many countries makes interpretation of this variation difficult.

**Impact on human health**

Person-to-person spread of salmonellas may be common in developing countries but is rare in developed countries, where most infections are food-borne. Fluoroquinolones are important drugs for the treatment of extra-intestinal infections caused by *Salmonella*.

Prior use of antibiotics in humans increases the likelihood of contracting salmonellosis caused by susceptible but especially resistant strains.

The common serotypes of *Salmonella* cause bacteraemia in 0.5 - 2.5% of culture-confirmed salmonellosis cases in the UK and in less than 6% in the USA. Untreated or ineffectively treated salmonella bacteraemia in humans can be fatal. There has been only one published case of a non-fatal infection by *S. typhimurium* DT204c infection due to a resistant strain of animal origin that failed fluoroquinolone therapy. There is concern, however, that zoonotic salmonellae with decreased susceptibility to fluoroquinolones are increasing and that a small proportion of these will cause invasive infections that require treatment, possibly with a fluoroquinolone, and that treatment failure could occur. The emergence of extended-spectrum beta-lactamase-producing salmonellae in some locales also increases this concern because of limited treatment options.

Although there is no microbiologically proven link between antibiotic resistance and virulence for humans in zoonotic salmonella, increased rates of hospitalisation have been reported for patients with infections with multiresistant *S. typhimurium* DT104.

Prolonged carriage of fluoroquinolone-resistant salmonella has implications for food handlers and health care workers, such as delay in return to work.
Conclusions on links between quinolone use in food animals and observed increases in quinolone-resistant foodborne pathogens and human treatment problems:

The use of fluoroquinolones in food animals has led to the emergence of fluoroquinolone-resistant Campylobacter and of Salmonella with reduced susceptibility to fluoroquinolones.

There has been little documented impact of this resistance on human health to date, but there is concern about the potential human health consequences if resistance were to increase and spread. Further research and data gathering are essential to quantify this potential.
IV. Quinolone use in animals and resistance in animal bacteria

Quinolone use in animals

Nalidixic acid was developed in the early 1960’s and was the first quinolone used clinically in animals. Subsequent quinolones, all congeners of nalidixic acid and synthesised in the late 1960s and 1970s, clearly showed both improved antibacterial and pharmacokinetic properties as well as reduced side effects. Some of these are still used in veterinary medicine (flumequine, oxolinic acid) in a limited number of countries. Clinically, a significant breakthrough was achieved by the introduction of the fluoroquinolones. Currently, several fluoroquinolones are available for therapy of animals in many countries. However, the usage of these fluoroquinolones differs greatly as regards animal species, indications, label indications, and geographic spread, which are summarized in Table 1 and Table 2.

The data presented indicate that quinolones are used for treatment of animal disease in many countries of the world and in some regions they are also used for disease prevention. Table 1 is not able to show that in some regions of the world there are significant amounts of generic and/or counterfeit drugs used, sometimes of unknown or variable product quality. The exact amount of this use is difficult to quantify as the usage data are not available, but it is believed to exceed the use of veterinary proprietary product(s) worldwide.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Trade name</th>
<th>cattle</th>
<th>swine</th>
<th>chickens</th>
<th>turkeys</th>
<th>Dogs</th>
<th>cats</th>
<th>fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>enrofloxacin</td>
<td>Baytril</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X*</td>
</tr>
<tr>
<td>danofloxacin</td>
<td>Advoxin,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Advocid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>norfloxacin</td>
<td>Quinubic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ofloxacin</td>
<td>Oxaldin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>generic</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sarafoxacin</td>
<td>Floxasol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saraflox</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sarafin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>orbifloxacin</td>
<td>Victas</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orbax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marbofloxacin</td>
<td>Marbocyl</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flumequine</td>
<td>many</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxolinic acid</td>
<td>many</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>difloxacin</td>
<td>Vetequinon</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dicural</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Baytril is not licensed for this use but generic products are available in some countries
Furthermore, limited usage data from developed countries have been available to regulatory authorities in the form of quantity (by weight) used in some countries. More specific information on the distribution of use by region, animal species and type, indication and formulation has traditionally not been available because pharmaceutical companies have tended to view these data as proprietary information. Efforts are being made in some regions to at least partially overcome this problem by third party collection and tabulation of usage data in a manner that protects confidentiality. This data collection may not, however, address the use of generic quinolones.

Although the major sources of food-borne salmonella and campylobacter are livestock and poultry, it is recognized that companion and feral animals exposed to quinolone selective pressure represent a potential source of anti-microbial resistant zoonotic bacteria for food animals, and pets are a potential source of infection for humans through direct contact. The first isolations for Salmonella typhimurium DT104 in the UK were from non-domestic birds and human beings. Increasingly, this zoonotic pathogen has been

<table>
<thead>
<tr>
<th>Region</th>
<th>Livestock</th>
<th>Poultry</th>
<th>Pet animals</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>enrofloxacin, flumequine, marbofloxacin, danofloxacin</td>
<td>enrofloxacin, difloxacin, flumequine, oxolinic acid</td>
<td>enrofloxacin, difloxacin, marbofloxacin</td>
<td>sarafloxacin, (oxolinic acid) *</td>
</tr>
<tr>
<td>USA</td>
<td>none</td>
<td>enrofloxacin, sarafloxacin</td>
<td>enrofloxacin, difloxacin, orbifloxacin</td>
<td>none</td>
</tr>
<tr>
<td>Japan</td>
<td>enrofloxacin, danofloxacin, orbifloxacin, difloxacin, oxolinic acid</td>
<td>enrofloxacin, danofloxacin, ofloxacin, vebufloxacin, oxolinic acid</td>
<td>enrofloxacin, orbifloxacin</td>
<td>oxolinic acid</td>
</tr>
<tr>
<td>Asia</td>
<td>enrofloxacin, danofloxacin, ciprofloxacin</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, ofloxacin, flumequine, norfloxacin, oxolinic acid, (sarafloxacin)</td>
<td>enrofloxacin</td>
<td>oxolinic acid, flumequine</td>
</tr>
<tr>
<td>Latin America</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, norfloxacin, (flumequine)</td>
<td>enrofloxacin, ciprofloxacin, danofloxacin, norfloxacin, (flumequine, oxolinic acid)</td>
<td>enrofloxacin</td>
<td>oxolinic acid</td>
</tr>
<tr>
<td>Canada</td>
<td>enrofloxacin b</td>
<td>enrofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>none</td>
<td>none</td>
<td>enrofloxacin</td>
<td>none</td>
</tr>
<tr>
<td>South Africa</td>
<td>enrofloxacin, danofloxacin</td>
<td>enrofloxacin, danofloxacin</td>
<td></td>
<td>enrofloxacin</td>
</tr>
</tbody>
</table>

* Substances in parentheses are in limited use.

b Voluntarily withdrawn from the market in 1998.
isolated from faeces of farm animals and in some countries is now endemic in these species, and it has been isolated from companion and feral animals as well. Thus, companion animal data are presented in these tables with information from food animals.

Further, the potential importance of extra label use in other species, including horses, is recognized. In some countries, veterinarians have the authority to use these products in other (extra label) indications and situations; elsewhere, veterinarians are restricted to the use of these products to label indications or specifically when no other medicinal products are available for that disease in that species. Finally, there are many developing countries where these products may be used by producers without the requirement of veterinary involvement.

As indicated in Table 3, quinolones are licensed for treatment of *Salmonella* infections in some animal species. This is noteworthy because treatment of salmonellosis with antibiotics has been shown to prolong carriage of salmonella in food animals, however

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Licensed use</th>
<th>Major bacteria</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>cattle</td>
<td>respiratory, enteric</td>
<td><em>Pasteurella</em> spp., <em>Haemophilus somnus</em>, <em>Mycoplasma bovis</em></td>
<td>injectable, bolus</td>
</tr>
<tr>
<td>swine</td>
<td>respiratory, enteric, mastitis/metritis</td>
<td><em>Pasteurella</em> spp., <em>Actinobacillus pleuropneumoniae</em>, <em>Mycoplasma</em>, <em>E. coli</em></td>
<td>injectable, oral solution, feed medication</td>
</tr>
<tr>
<td>broilers</td>
<td>respiratory, enteric</td>
<td><em>E. coli</em>, <em>Mycoplasma</em>, <em>Pasteurella</em>, <em>Salmonella</em></td>
<td>oral (water medication)</td>
</tr>
<tr>
<td>turkeys</td>
<td>respiratory, enteric</td>
<td><em>E. coli</em>, <em>Mycoplasma</em>, <em>Pasteurella</em>, <em>Salmonella</em></td>
<td>oral (water medication)</td>
</tr>
<tr>
<td>fish</td>
<td>generalised conditions (septicemia), skin/ulcers</td>
<td><em>Aeromonas hydrophila</em>, <em>Vibrio</em> spp.</td>
<td>oral (feed medication), water bath</td>
</tr>
<tr>
<td>dogs</td>
<td>skins/wounds, urinary tract, respiratory</td>
<td><em>S. intermedius</em>, <em>E. coli</em>, <em>Pasteurella</em></td>
<td>tablets, injectable</td>
</tr>
<tr>
<td>cats</td>
<td>skins/wounds, urinary tract, respiratory</td>
<td><em>S. intermedius</em>, <em>E. coli</em>, <em>Pasteurella</em></td>
<td>tablets, injectable</td>
</tr>
</tbody>
</table>

Use of Quinolones in Food Animals and Potential Impact on Human Health 11
to date this has not been shown to occur with quinolone use. There are no published data regarding the incidence of treatment failures of salmonellosis in animals caused by any strain of *Salmonella* that has reduced susceptibility (distinguished from clinical resistance) to fluoroquinolones, however there have been treatment failures in calves due to clinically resistant *Salmonella* in Germany.

**Licensing and post-licensing procedures and requirements for quinolones in various parts of the world**

Considerable variation in the regulatory processes of different countries is recognized to occur. Licensing requirements range from highly controlled to minimal scrutiny and in some cases the products are available without a review process by a regulatory authority. In some countries there is no legal framework for prescription of veterinary pharmaceuticals.

The Veterinary International Cooperation on Harmonisation (VICH) is an international initiative intended to harmonise the regulatory requirements for registration among the United States, the European Union and Japan. This initiative is held under the auspices of the Office International des Epizooties (OIE). The issue of antimicrobial resistance has not been posed to the VICH, although it could be.

Some regulatory bodies have in place requirements to include the evaluation of microbiological safety in terms of resistance to antimicrobials. Technical areas required to be addressed in drug licensing in the United States, the European Union and Japan include target animal efficacy and safety, human food safety regarding drug residues, and product quality. Increasingly, countries are concerned about the emergence of resistance among zoonotic pathogens. A fundamental addition to the registration process in some countries is the evaluation of resistance concerns pre-approval and the monitoring of susceptibility of zoonotic and/or target animal pathogens post-approval as a critical part of the registration process. In some jurisdictions, issues arising for a particular product have to be addressed before renewal of the marketing authorisation. Furthermore, many groups are initiating efforts to develop principles of prudent use and to educate veterinarians and end-users about the prudent or judicious use of antimicrobials.

**Quinolone resistance patterns in animal pathogens and commensals**

Resistance to quinolones, in the form of nalidixic acid resistance and reduced susceptibility or clinical resistance to fluoroquinolones, has been observed in target pathogens and commensals. The acquisition of resistance has been found to develop through step-wise, chromosomal mutation, and to be directed towards both the target enzymes and the efflux pump of the bacteria.
There are vast regional differences in monitoring of the resistance patterns in animal pathogens and commensals. The majority of regions have no monitoring programmes ongoing. There are also substantial differences in the types of samples that are collected for monitoring, including clinical submissions, slaughterhouse samples, and samples collected from herds/farms/hatcherries. These are in some cases collected on an ongoing basis and in other cases are compiled for a specific period of time and for a specific purpose, such as a regional or national survey. Even with the ongoing monitoring programmes that do exist, the practising veterinarian or other user is unable to obtain easily the relevant data on a regional and national basis. Government bodies, veterinarians, pharmaceutical companies and the food-producing industry may be conducting independent monitoring programmes, but efforts and procedures differ, resulting in data from different laboratories and countries that in some (perhaps most) cases cannot be compared. This same dilemma exists when attempting to compare data from different regions.

In addition to monitoring programmes as a source of resistance data, in many countries there are private or public animal disease diagnostic laboratories that conduct bacterial culture and antimicrobial susceptibility analyses on clinical submissions. Veterinarians use this information to evaluate the need for quinolone treatment of animals. Furthermore, regulatory agencies may use some of these data or specimens (especially of zoonotic agents) to assess the emergence and transmission of resistance in zoonotic food-borne bacteria.
V. Recommendations

Research needs

Applied research should be conducted:

- to further evaluate the impact of bacteria with reduced susceptibility to fluoroquinolones on the outcome of treatment of human infections
- on established and novel mechanisms of resistance to quinolones in food-borne pathogens
- on emergence and dissemination of quinolone resistance in zoonotic bacteria and its spread within and between species. Specific research subjects would be the spread of fluoroquinolone-resistant bacteria from animals to farm workers and the establishment of the clonal relationships between quinolone-resistant strains in animals and humans
- on the persistence of quinolone-resistant strains compared to susceptible strains of Campylobacter and Salmonella, particularly in food animals
- on methods and tools that would reduce the risk of selecting bacteria with quinolone resistance or reduced susceptibility in animals and that would lead to the restoration of susceptible flora after resistance. This would include research into:
  - the appropriate dose, route and duration of administration of quinolones in animals. In particular, studies are needed on the relative risks of dosing via water and feed in comparison to other routes
  - a broad range of animal production and management practices.
  Similar studies to evaluate the effects of reduction of use of quinolones on restoration of susceptible flora are also needed.
- to develop economical alternatives to the use of antimicrobials for disease prevention (e.g., vaccines, probiotics, competitive exclusion principles, etc.) and evaluate the impact that these alternatives might have on selection for resistance
- to evaluate the impact of the use of quinolones in domestic pets and birds on the introduction, development, and persistence of resistant bacteria in the farm environment
- on the epidemiology of food-borne pathogens.

In addition, there is a critical need to investigate methods and procedures to appropriately address resistance concerns that arise prior to licensing of quinolones. This research should define the appropriate risk assessment models and data needed to allow the models to be implemented. Additionally, the most appropriate post-approval monitoring schemes should be developed which complement the pre-approval risk assessment models.
Data gathering needs

Gathering of surveillance data on resistance levels and drug usage is critically important for assessments of risk. Specifically, there are five purposes to monitoring occurrence of resistance in zoonotic pathogens, and these are to:

- assist in clinical decisions
- provide information for the development of new drugs
- target and evaluate prevention and control measures (including prudent antimicrobial use) and epidemiology
- identify trends in antibiotic resistance patterns
- inform public policy with information for risk assessment and antimicrobial use.

Quinolone usage data should be accessible to the regulatory authorities to help interpret the related resistance surveillance data.

Monitoring of antimicrobial resistance

- In order to generate more reliable and comparable data, it is recommended that there be international co-ordination on surveillance methods and data exchange.

- The recommendations for surveillance methodologies as outlined in the Berlin document (proper reference) are re-emphasized.

- Resistance monitoring should be targeted to include at least *E. coli*, Salmonella, and Campylobacter isolated from animals.

- Laboratory susceptibility testing techniques, including breakpoints used in public health and food chain monitoring, should be standardised with a view to provide comparable data.

- Surveillance should ideally be structured to allow elements of both local monitoring and reporting as surveillance systems often pool data regionally or nationally. Thus, problems occurring at the level of the individual farm that are important for control of local resistance can be masked.

- Research should be undertaken to determine the optimum surveillance sampling schemes required to fulfil the objectives of the monitoring programme (i.e., to detect resistance emergence vs. to monitor the safety of the food supply).

- Components for monitoring of resistance
  - national quality control
  - international standardisation and co-ordination of methods of susceptibility testing.

- For resistance to fluoroquinolones, quantitative susceptibility data and categorical nalidixic acid resistance data are more sensitive for the detection of common first-step mutations that cause reduced susceptibility than are categorical fluoroquinolone resistance data.
Usage of quinolones in food animals

There is a need to acquire more and better information on quinolone usage in food animals. Information should also include generic drug use. Documentation of total usage at the national level by species, product class, indications, dose and geographical region is a first step. Availability of these data is critical for more accurate risk assessment. Ideally these data should be linked to epidemiological investigations. The more specific the consumption data, the better the ability to interpret any changes in resistance. If necessary, governments could either handle these data in a confidential and proprietary manner from the drug sponsor, or could survey end-users regarding prescribing practices or even require submission of consumption and usage data.

Prudent use of antimicrobials in livestock

As an element to further encourage activities for the prudent use of antimicrobials in livestock WHO should take leadership with the Food and Agriculture Organization (FAO) and the OIE to convene, in collaboration with other international and intergovernmental organisations, an expert consultation to develop a code of practice for prudent use of antimicrobials in food animal production. WHO should ensure that public health safeguards are considered in the development of the code of practice.

The following proposals of the participants of this meeting should be considered at such a consultation:

- Veterinarians should have at their disposal antibiotics to treat sick animals, which in certain instances may include quinolones. Quinolones, as with any antimicrobial agent, should never be used as a substitute for good animal husbandry practices. Quinolones should be administered in accordance with prudent use principles. Prudent use of quinolones is defined as the practices that maximize therapeutic effect while minimising the emergence of resistance.

- Member States should be encouraged to promote prudent use of quinolones in veterinary medicine. Specifically for quinolones, prudent use includes, but is not limited to:
  (a) treatment only under close supervision of a veterinarian for animals under his/her care with written records of use
  (b) treatment upon a diagnosis based, whenever possible, on bacterial culture and susceptibility testing with the encouragement of accurate on-farm record-keeping
  (c) when culture and susceptibility culture results are known, an efficacious narrow-spectrum antibiotic is preferable for therapy over quinolones.
• Education of veterinarians and end-users about prudent use principles is critical to their implementation and should cover the risks of selecting resistant bacteria, the fundamental understanding of antimicrobials and prudent use concepts. Veterinary educators should be encouraged to ensure that these prudent use principles are included in veterinary curricula.

• Registration of quinolones should be only for therapeutic use and not for performance enhancement. They should be registered only as prescription veterinary pharmaceuticals, with appropriate enforcement of that requirement.

• No quinolones should be administered to a food animal unless the product has been evaluated and authorised by competent authorities, including a thorough assessment which considers the potential for development of resistance that may affect public health, and encouraging a post-approval monitoring programme to detect trends toward the emergence of resistance of public health significance. Authorities should be encouraged to utilize the data collected through such a monitoring programme to take measures to mitigate the development of resistance.

• Use of quinolones in food animals other than the licensed indications for use should be discouraged.

Aquaculture

Quinolone use in aquaculture was not discussed at this consultation. Because of the unique use of quinolones in aquaculture, investigation of the public health impact of this use is critical and should be facilitated by WHO.

Notes


2. *This term is used in reference to all generations of quinolones, including fluoroquinolones.*

3. *Information from working paper number 9, "Overview of quinolone usage for food-producing animals" submitted by van Diest and de Jong.*
LIST OF PARTICIPANTS AND OBSERVERS

Dr F. Aarestrup
Danish Veterinary Laboratory
Bülowsvej 27
Copenhagen
Denmark
Tel: 45 35 01 00
Fax: 45 35 01 20
E-mail: faa@svs.dk

Professor J. Acar
Service de Microbiologie médicale
Hôpital Saint Joseph
185, rue Raymond Loosserand
F-Paris 75674 Cedex 14
France
Tel: 33 1 44 12 33 66
Fax: 33 1 44 12 34 93

Dr F. Angulo
Centers for Disease Control and Prevention
USDA/FAO/CDC Foodborne Diseases Project
1600 Clifton Road
Atlanta, GA 30333
USA
Tel: 1 404 329 9854
Fax: 1 404 639 2205
E-mail: fda@cdc.gov

Dr F. Baquero
Hospital Ramón y Cajal
C. Colmenar Km. 9,102
Madrid 28034
Spain
Tel: 34 1 336 8330
Fax: 34 1 336 9016
E-mail: fernando.baquero@hrc.es

Dr D. Beil
National Center for Infectious Diseases
Centers for Disease Control and Prevention
1600 Clifton Road, NE
Atlanta, GA 30333
USA
Tel: 1 404 639 2603
Fax: 1 404 639 4197
E-mail: dmb1@cdc.gov

Dr M. Bland
3502 Jomar Drive
Napa, CA 94558
USA
Tel: 1 707 257 1916
E-mail: mcbland@pacsbell.net

Dr J. Boissau
L'Agence Nationale du Médicament Vétérinaire
La Haute Marche
Javènes 35 133 Fougères
France
Tel: 33 2 954 7378
Fax: 33 2 954 7889
E-mail: varlo@salva.net

Dr T. Burkgren
American Association of Swine Practitioners (AASP)
902 1st Street
Parry, IA 50220
USA
Tel: 1 515 465 4525
Fax: 1 515 465 3832
E-mail: aasp@netins.net

Professor Y. Chong
Department of Clinical Pathology
Yonsei University College of Medicine
Severance Hospital
Seoul
Republic of Korea
Tel: 82 7 361 5662
Fax: 82 2 313 0656
E-mail: leekcpp@yumc.yonsei.ac.kr

Dr R. Carnevale
Representative, Commission mondiale de l'industrie de la santé (COMISA)
Animal Health Institute
501 Wyth Street
Alexandria, VA 22314-1917
USA
Tel: 1 703 884 0011
E-mail: rcarnevale@ahin.org

Dr Paula Fedorka-Cray
Poultry Micro Research Unit
USDA-ARS Richard Russell Research Center
950 College Station Road
Athens, GA 30605-2720
USA
Tel: 1 706 546 3305
Fax: 1 706 546 3066
E-mail: pcray@ars.usda.gov

Dr A. Flahaut
Unité de Recherches U.444 INSEM
27, rue Chaliguy
75751 Paris Cedex 12
France
Tel: 33 1 56 01 68 13
Fax: 33 1 40 30 68 14
E-mail: flahaut@53e.jussieu.fr

Professor H. Goossens
Chairperson
Department of Microbiology
Université Libre de Bruxelles
V/Wijstraat 10
Antwerp B-2650
Belgium
Tel: 32 3 821 3789
Fax: 32 3 825 4281
E-mail: herman.goossens@uclouvain.be

Professor H. Goossens
Chairperson
Department of Microbiology
Université Libre de Bruxelles
V/Wijstraat 10
Antwerp B-2650
Belgium
Tel: 32 3 821 3789
Fax: 32 3 825 4281
E-mail: herman.goossens@uclouvain.be

Dr R. Helmuth
Federal Institute for Health Protection of Consumers and Veterinary Medicine
National Salmonella Reference Laboratory
PO Box 480447
12254 Berlin
Germany
Tel: 49 30 6142 2233
Fax: 49 30 6142 3003
E-mail: r.helmuth@bvgv.de

Dr D. Hooper
Chairperson
Infectious Disease Division
Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114
USA
Tel: 1 617 726 3812
Fax: 1 617 726 7416
E-mail: dhooper@partners.org

Professor M. Inoue
Department of Microbiology
Kitasato University School of Medicine
1-15-1 Kitasato, Sagamihara
Kanagawa 228-855
Japan
Tel: 81 427 78 8111
Fax: 81 427 78 8111
E-mail: matsum@kitasato-u.ac.jp

Professor H. Koornhof
Department of Clinical Microbiology and Infectious Diseases
South African Institute for Medical Research
Hospital Street
PO Box 1038
Johannesburg 2000
South Africa
Tel: 27 11 469 9330
Fax: 27 11 469 9332
E-mail: dchooper@partners.org

Professor H. Koornhof
Department of Clinical Microbiology and Infectious Diseases
South African Institute for Medical Research
Hospital Street
PO Box 1038
Johannesburg 2000
South Africa
Tel: 27 11 469 9330
Fax: 27 11 469 9332
E-mail: dchooper@partners.org

Dr K. Lechtenberg
Midwest Veterinary Services, Inc.
1443 Highway 77
Oakland, NE 68045-5515
USA
Tel: 1 402 685 6502
Fax: 1 402 685 6008
E-mail: mms.inc@necis.net

Dr Diane Lightfoot
Microbiological Diagnostic Unit
University of Melbourne
Royal Parade
Parkville, Victoria 3052
Australia
Tel: 61 3 9344 5701
Fax: 61 3 9344 7833
E-mail: Diane_Lightfoot. MDU@unimelb.edu.au

Dr S. McEwen
Rapporteur
University of Guelph
Food Safety Epidemiology
Department of Population Medicine
Guelph N1G 2W1
Canada
Tel: 519 824 4120
ext 4751
Fax: 519 763 3117
E-mail: smcswen@ovcnet.uoguelph.ca

Dr P. McMillin
British Veterinary Laboratory
Association
Main Site Lane
Dalton, Thirsk Y07 3JA
North Yorkshire
UK
Tel: 44 1845 577907
Fax: 44 1845 577978
E-mail: PaulMcMillin@compuserve.com

Dr Laura Piddock
Department of Infection Antimicrobial Agents Research Group
University of Birmingham
Birmingham B15 2TT
United Kingdom
Tel: 44 121 414 6969
Fax: 44 121 414 6966
E-mail: ljpiddock@bham.ac.uk

Use of Quinolones in Food Animals and Potential Impact on Human Health

19
Use of Quinolones in Food Animals and Potential Impact on Human Health

Professor M. Wierup
Swedish Animal Health Service
Johanneshov 121 86
Sweden
Tel: 46 8 725 80 00
Fax: 46 8 725 81 72
E-mail: Martin.Wierup@globen.postnet.se

Dr. S. Wong
Tan Tock Seng Hospital
Communicable Disease Centre
Moulmein Road
Singapore 308433
Tel: 65 3596501
Fax: 65 2524056
E-mail: Sin_Yew_Wong@nus.edu.sg

Dr. H. Yoshimura
Antibiotic Department
National Veterinary Assay Laboratory
Ministry of Agriculture, Forestry and Fisheries (MAFF).
Tokyo Japan
Fax: 81 423 217 169
E-mail: yoshimur@gvilv.niv.go.jp

Dr. J. van Diest
Representative, COMISA
Bayer Animal Health
D-51368 Leverkusen
Germany
Tel: 49 2173 48 832
Fax: 49 2173 48 832
E-mail: Jan.Diest@bayer-ag.de

Dr H.C. Wegener
National Veterinary Laboratory
27 Bülowsvej
Copenhagen V
1790 Denmark
Tel: 45 35 30 01 54
Fax: 45 35 30 01 20
E-mail: hcv@svd.dk

Dr A. Bryskier
International Federation of Pharmaceutical Manufacturers Associations (IFPMA)
102 route de Noisy
93235 Romainville Cedex
France
Tel: 33 (1) 49 91 51 21
Fax: 33 (1) 49 91 50 20
E-mail: andre.bryskier@hmr.com

Dr. T. Daskaleros
Directorate-General XXIV
European Commission
Rue de la Loi 200
B-1049 Brussels
Belgium
Tel: 32 2 295 67 74
Fax: 32 2 296 299
E-mail: panagiotis.daskaleros@dg24.coebe

Dr. P. Jones
European Agency for the Evaluation of Medicinal Products
7 Westhley Circus, Canary Wharf
London E14 4HB
United Kingdom
Tel: 44 171 418 8400
Fax: 44 171 418 8447
E-mail: peter.jones@emea.edra.org

Dr. J. Paakkonen
Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla
00110 Rome
Italy
Tel: 39 6 570 53523
Fax: 39 6 570 54593
E-mail: juhani.paakkonen@fao.org

Dr. Barbara Röstel
Office International des Epizooties
CNEVA - Fougeres
B. P. 203
35302 Fougeres
France
Tel: 33 296 94 78 95
Fax: 33 296 94 78 99
E-mail: vaf010@calvicos.com

Dr. R. Bywater
Representative, COMISA
Europe House
Bancroft Road, Reigate
Surrey, RH2 7RP
UK
Tel: 44 1737 227 402
Fax: 44 1737 227 410
E-mail: Bywater00@pfizer.com

Dr. Gail Cassell* American Society of Microbiology
1325 Massachusetts Avenue
Washington, DC 20005 USA
Tel: 202 242 2077
E-mail: cassell_gail_h@illy.com

Dr. P. Choraine
Federation of Veterinarians of Europe
Avenue Fonsny 41
B-1060 Brussels
Belgium
Tel: 32 2 538 29 63
Fax: 32 2 537 28 28
E-mail: Pierre.Choraine@emea.edra.org

Dr. T. Gomez
United States Department of Agriculture (USDA)
Animal and Plant Health Inspection Service (APHIS)
Veterinary Services Centers for Disease Control and Prevention
1600 Clifton Road, NE
Atlanta, GA 30333 USA
Tel: 1 404 639 3233
Fax: 1 404 639 2212
E-mail: tmg1@cdc.gov

Observers

Dr S. Brown
American Academy of Veterinary Pharmacology and Therapeutics
301 Henrietta Street
Kalamazoo, MI 49007 USA
Tel: 1 616 833 2412
Fax: 1 616 833 7721
E-mail: scott.a.brown@an.psu.edu

Dr. R. Bywater
Representative, COMISA
Europe House
Bancroft Road, Reigate
Surrey, RH2 7RP
UK
Tel: 44 1737 227 402
Fax: 44 1737 227 410
E-mail: Bywater00@pfizer.com

Dr. Gail Cassell* American Society of Microbiology
1325 Massachusetts Avenue
Washington, DC 20005 USA
Tel: 202 242 2077
E-mail: cassell_gail_h@illy.com

Dr. P. Choraine
Federation of Veterinarians of Europe
Avenue Fonsny 41
B-1060 Brussels
Belgium
Tel: 32 2 538 29 63
Fax: 32 2 537 28 28
E-mail: Pierre.Choraine@emea.edra.org

Dr. T. Gomez
United States Department of Agriculture (USDA)
Animal and Plant Health Inspection Service (APHIS)
Veterinary Services Centers for Disease Control and Prevention
1600 Clifton Road, NE
Atlanta, GA 30333 USA
Tel: 1 404 639 3233
Fax: 1 404 639 2212
E-mail: tmg1@cdc.gov

Representatives from other International Organizations

Dr N.-O. Bjørregaard
World Veterinary Association
Rosenlund Aale 8
DK-2720 Vanlose
Denmark
Tel: 45 38 71 01 56
Fax: 45 38 71 03 22
E-mail: wva@dcd.dk

Dr. A. Bryskier
International Federation of Pharmaceutical Manufacturers Associations (IFPMA)
102 route de Noisy
93235 Romainville Cedex
France
Tel: 33 (1) 49 91 51 21
Fax: 33 (1) 49 91 50 20
E-mail: andre.bryskier@hmr.com

Dr R. Stevenson
International Pig Veterinary Society
The Veterinary Centre
Porth Y Carne Street
Usk, Gwent
UK
Tel: 44 1291 872637
Fax: 44 1291 873940

20 Use of Quinolones in Food Animals and Potential Impact on Human Health
Dr. Rebecca Irwin  
Health Canada  
Guelph Laboratory  
110 Stone Road West  
Guelph, Ontario, N1G 3W4  
Canada  
Tel: 1 519 822 3300, Ext. 256  
Fax: 1 519 822 2280  
E-mail: rebecca_irwin@hc-sc.gc.ca

Dr. P. MacDonald  
Canadian Food Inspection Agency  
Animal Health and Production  
59 Camelot Drive  
Nepean, Ontario, K1A 0Y9  
Canada  
Tel: 1 613 225 2342 Ext. 5327  
Fax: 1 613 228 6614  
E-mail: p.mcdonald@em.agr.ca

Dr. Odette Morin  
IFPMA  
30 rue de St. Jean  
Geneva 1211  
Switzerland  
Tel: 41 22 340 1200  
Fax: 41 22 340 1380  
E-mail: o.morin@ifpma.org

Dr. Y. Oketani  
Office of Veterinary Drug Administration  
Ministry of Agriculture, Forestry and Fisheries  
1-2-1 Kasumigaseki  
Chiyoda-ku, Tokyo 100  
Japan  
Tel: 81 3 3591  
Fax: 81 3 3506 2546

Dr. Sharon Thompson  
United States Food and Drug Administration  
Center for Veterinary Medicine  
7500 Standish Place  
Rockville, MD 20855  
USA  
Tel: 1 301 594 1798  
Fax: 1 301 594 1830  
E-mail: s.thompson@bangate.fda.gov

Dr. Linda Tollefson  
United States Food and Drug Administration  
Center for Veterinary Medicine  
Office of Surveillance and Compliance  
Rockville, MD 20855  
USA  
Tel: 1 301 594 1760  
Fax: 1 301 594 4512  
E-mail: l.tollefson@bangate.fda.gov

Dr. L. Vogel  
American Veterinary Medical Association  
1931 N. Meacham Road  
Suite 100  
Schaumburg, IL 60173-4360  
USA  
Tel: 1 847 825 8070  
Fax: 1 847 825 1326  
E-mail: lvogel@avma.org

Dr. C. Verschueren  
COMISA  
Rue Defacqz, 1/Bte 8  
1000 Brussels  
Belgium  
Tel: 32 2 541 0111  
Fax: 32 2 541 0119  
E-mail: comisa@fedesa.be

Mr. Y. Yano  
Japanese Veterinary Pharmaceutical Association (JVPA)  
Bld-1, 2-Kanda  
Sungadai, Chiyoda-ku  
Tokyo 101  
Japan  
Tel: 81 3 3294 3243  
Fax: 81 3 3294 0084

WHO Secretariat  

Dr. F.X. Meslin  
Chief, Zoonotic Diseases  
Division of Emerging and other Communicable Diseases  
World Health Organization  
CH-1211 Geneva 27  
Switzerland  
Tel: 41 22 791 2675  
Fax: 41 22 791 4893  
E-mail: meslin@who.ch

Mr. A. Reilly  
Programme of Food Safety and Food Aid  
World Health Organization  
CH-1211 Geneva 27  
Switzerland  
Tel: 41 22 791 3462  
Fax: 41 22 791 4807  
E-mail: reillya@who.ch

Dr. K. Stähler  
Secretary, Zoonotic Diseases  
Division of Emerging and other Communicable Diseases  
World Health Organization  
CH-1211 Geneva 27  
Switzerland  
Tel: 41 22 791 2529  
Fax: 41 22 791 4893  
E-mail: stahler@who.ch

Dr. Rosemund Williams  
Bacterial Diseases  
Division of Emerging and other Communicable Diseases  
World Health Organization  
CH-1211 Geneva 27  
Switzerland  
Tel: 41 22 791 4144  
Fax: 41 22 791 4878  
E-mail: williams@who.ch

Use of Quinolones in Food Animals and Potential Impact on Human Health  
21

* Unable to attend
AGENDA AND TIMETABLE

Tuesday, 2 June 1998

<table>
<thead>
<tr>
<th>TIME</th>
<th>SUBJECT</th>
<th>SPEAKER</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:30</td>
<td>Opening, Introductions</td>
<td>David Heymann, WHO</td>
</tr>
<tr>
<td>13:00</td>
<td>Overview of Quinolone Use and Resistance in Humans</td>
<td></td>
</tr>
<tr>
<td>13:20</td>
<td>Mechanisms of quinolone resistance</td>
<td>David Hooper, USA</td>
</tr>
<tr>
<td>13:25</td>
<td>Discussion</td>
<td>Claude-James Soussy, France</td>
</tr>
<tr>
<td>13:45</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>13:50</td>
<td>Review of the clinical use of quinolones in human medicine: Western Hemisphere</td>
<td>David Hooper, USA</td>
</tr>
<tr>
<td>14:00</td>
<td>Review of the clinical use of quinolones in human medicine: Eastern Hemisphere</td>
<td>Sin-Yew Wong, Singapore</td>
</tr>
<tr>
<td>14:10</td>
<td>Discussion</td>
<td>Hendrik Koornhof, South Africa</td>
</tr>
<tr>
<td>14:15</td>
<td>Quinolone resistance in community-acquired pathogens in South Africa</td>
<td></td>
</tr>
<tr>
<td>14:25</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>14:30</td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td>15:00</td>
<td>Resistance to quinolones in zoonotic bacteria in China</td>
<td>Shaohong Jin, China</td>
</tr>
<tr>
<td>15:10</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>15:15</td>
<td>Review of quinolone resistance in human pathogens</td>
<td>Yunso P Chong, Republic of Korea</td>
</tr>
<tr>
<td>15:35</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>15:40</td>
<td>Quinolones in the environment</td>
<td>Norbert Schmeer, Germany</td>
</tr>
<tr>
<td>15:50</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>15:55</td>
<td>General Discussion</td>
<td></td>
</tr>
<tr>
<td>16:15</td>
<td>Production, Licensing and Use of Quinolones for Food Animals</td>
<td></td>
</tr>
<tr>
<td>16:15</td>
<td>Overview of quinolone production for food animal usage</td>
<td>Jan van Diest, Representative of COMISA</td>
</tr>
<tr>
<td>16:25</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>16:30</td>
<td>Licensing of quinolones *</td>
<td>Jacques Boisseau, France *</td>
</tr>
<tr>
<td>16:40</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>16:45</td>
<td>The authorisation of antimicrobials in the EU for veterinary use</td>
<td>Peter Jones, Representative of EMEA</td>
</tr>
<tr>
<td>17:00</td>
<td>Discussion</td>
<td></td>
</tr>
<tr>
<td>17:05</td>
<td>Quinolone licensing for use in food animals and related problems facing regulators in the USA</td>
<td>Stephen Sundlof, USA</td>
</tr>
</tbody>
</table>
Tuesday, 2 June 1998 cont.

17:20 Discussion
17:25 General discussion
17:45 Adjourn for the day

Wednesday, 3 June 1998

PRODUCTION, LICENSING AND USE OF QUINOLONES FOR FOOD ANIMALS (cont.)

How and why are quinolones used in food animals?
— rationale, benefits and type of usage in various animals species and production steps/conditions

8:30 Examples of quinolone usage in poultry production in the USA
8:40 Quinolone usage in poultry medication in Europe
8:45 Discussion
8:50 Quinolone usage and resistant bacteria in chickens in Japan
9:00 Discussion
9:05 Examples of quinolone usage in poultry production in Thailand *
9:15 Discussion
9:20 Examples of quinolone usage in pig production in Latin America
9:30 Discussion
9:35 Review of quinolone usage in pig production
9:50 Discussion
9:55 Review of the use of quinolones in food animals
10:10 Discussion
10:15 General Discussion
10:30 Break

LINKS BETWEEN QUINOLONE RESISTANCE IN FOOD ANIMAL BACTERIA AND HUMAN DISEASE

Examples of in vitro quinolone resistance prevalence/trends in human and animal isolates of Food-borne Salmonella and Campylobacter.

11:00 Panel discussion and presentations: Diane LIGHTFOOT, Australia; Henrik WEGENER, Denmark; Reiner HELMUT, Germany; Matsuhisa INOUE, Japan **; Ellen STOBBERINGH, The Netherlands; Fernando BAGUERO, Spain *; Bertil KAISER, Sweden **; Sasitorn KANARAT, Thailand *; Laura PIDDOCK, UK; John THRELFA, UK; Fred ANGULO, USA; Paula FEDORKA-CRAY, USA.
13:00 Lunch
14:00 Panel discussion (continued)

Examples of in vivo problems (type and extent)

15:00 Overview of salmonellosis treatment problems
15:20 Discussion
15:30 Overview of campylobacteriosis treatment problems

Jacques ACAR, France
Herman GOOSSENS, Belgium
Wednesday, 3 June 1998 cont.

15:50 Discussion
16:00 Break
16:30 Quinolone use in agriculture and medical problems: How reliable are the data? Richard Carnevale, Representative of COMISA
16:50 Discussion
17:00 General Discussion

Risk Assessment

17:15 Risk assessment: data needs and methods Antoine Flahault, France
17:35 Discussion

Preparation for the Breakout Sessions

17:45 Challenge to the groups
18:15 Adjourn for the day

Thursday, 4 June 1998

8:30 Breakout Sessions
10:15 Break
10:45 Breakout Sessions (cont.)
12:30 Lunch
13:30 Breakout Sessions (cont.)
15:15 Break
15:45 Breakout Sessions (cont.)
17:00 First Plenary Session

Presentation of outlines of breakout session reports

18:00 Adjourn for the day

Friday, 5 June 1998

8:30 Final Plenary Session

Finalise conclusions and recommendations from the breakout session reports

10:00 Break
10:30 Final Plenary Session (cont.)
11:50 Concluding Remarks
12:00 Adjourn

* Paper not received, speaker unable to attend.
** Paper received, speaker unable to attend.
1 MECHANISMS OF QUINOLONE RESISTANCE

David C Hooper

Bacterial mechanisms of resistance to quinolones can be divided into two general categories, alterations in drug target enzymes and alterations in drug permeation that affect drug access to these target enzymes. As yet, no specific quinolone-modifying enzymes have been identified as causing resistance, although certain fungi are capable of degrading quinolones through metabolic pathways (7).

Modifications of drug target enzymes

DNA gyrase. DNA gyrase, one of two targets of quinolones, is an essential bacterial enzyme responsible for introducing negative superhelical twists into bacterial DNA (2,3). It is also capable of removing both negative and positive DNA superhelical twists. Negative supercoiling of DNA catalyzed by DNA gyrase is necessary for initiation of DNA replication, and positive supercoils that accumulate ahead of the DNA replication fork would impede fork propagation if not removed by DNA gyrase. The enzyme is composed of two A (GyrA) and two B (GyrB) subunits encoded by the gyrA and gyrB genes, respectively. Shortly after the discovery of Escherichia coli DNA gyrase, resistance to nalidixic acid was shown to be caused by mutations in the gyrA gene (4). Subsequently many studies in a wide range of gram-negative bacteria have identified amino acid changes in the GyrA and GyrB subunits that cause quinolone resistance or reductions in activity (5). These alterations result from single nucleotide changes in gyrA or gyrB that occur as spontaneous mutations and are selected by exposure to quinolones. There are some data indicating that quinolone exposure is mutagenic for bacteria and may thereby increase the frequency with which resistance mutations occur (6).

Resistance mutations have been clustered in the amino terminus [usually between amino acids 67 and 106 based on numbering in E. coli, the “quinolone-resistance-determining region (QRDR)] of GyrA near the active site tyrosine at position 122 (7). The two most common single sites of change are at positions 83 and 87. Quinolones bind specifically to the complex of DNA gyrase with DNA rather than DNA gyrase alone, and alterations at position 83 have been associated with reduced drug binding to this complex (8). The recently reported x-ray crystallographic structure of a fragment of GyrA localizes the QRDR to a positively charged surface along which DNA is thought to bind (9). Thus, a common model envisions that amino acid changes in the QRDR of GyrA alter the structure of the site of quinolone binding near the interface of the enzyme and DNA and that resistance is then caused by reduced drug affinity for the modified enzyme-DNA complex. Direct structural information on the site of quinolone binding within the complex is as yet lacking, however.
Alterations in the GyrB subunit also cause reductions in quinolone susceptibility but usually to a lesser degree than the most common GyrA mutations (10). These mutations have been clustered in the mid-portion of the GyrB amino acid sequence. There has been no reported crystallographic structure of GyrB that includes this region, but the homologous region of the crystal structure of yeast topoisomerase II enzyme is distant from the region homologous to the QRDR of GyrA (11), suggesting that this QRDR region of GyrB may not be directly involved in a putative quinolone binding site and that the molecular mechanism of resistance caused by alterations in GyrB may differ from that caused by alterations in GyrA.

Topoisomerase IV. Topoisomerase IV is also a quinolone target within bacterial cells. This enzyme, like DNA gyrase, is essential for DNA replication, but its role appears primarily to be the decatenation or unlinking of daughter chromosomes at the completion of a cycle of DNA replication to allow their segregation into daughter cells (12). Topoisomerase IV has a structure similar to that of DNA gyrase and is composed of two ParC (or GrlA in Staphylococcus aureus) and two ParE (GrlB in S. aureus) subunits (13,14). ParC is homologous to GyrA, and ParE is homologous to GyrB. Particularly highly conserved is the QRDR homologous region of ParC.

Resistance mutations in ParC similar to those in GyrA have been clustered in the equivalent QRDR region, with the most common mutations occurring at positions 80 and 84 (E. coli numbering) (13,15), although additional mutations outside this region near the active site have been described (16). Resistance mutations in ParE have also been identified in regions homologous to those causing resistance in GyrB (17). There have as yet been no studies of drug binding to topoisomerase IV-DNA complexes and no crystallographic structure of topoisomerase IV reported, but the similarities in overall subunit structure and amino acid sequence between topoisomerase IV and DNA gyrase suggest that the models of these two enzymes will be similar.

Relative roles of the two target enzymes in resistance and stepwise incremental resistance. In E. coli, genetic studies have clearly demonstrated that gyrA (and gyrB) mutations alone can cause quinolone resistance or reduced susceptibility. In contrast, parC (and parE) mutations alone have no effect on drug susceptibility (18). Double mutants with both gyrA and parC mutations, however, have higher levels of resistance than the same gyrA mutant alone. For S. aureus, this pattern is reversed. Mutations in grlA or grlB alone can cause quinolone resistance, and gyrA mutations only affect susceptibility when they occur together with grlA or grlB mutations (16,19). These patterns can be best understood in terms of the relative sensitivities of the two target enzymes to a given quinolone. In the case of E. coli, purified DNA gyrase is more sensitive to most quinolones than is purified topoisomerase IV, and the reverse is true for the two enzymes purified from S. aureus (20). Thus, mutations in the most sensitive target enzyme contribute to first-step resistance. Mutations in the less sensitive enzyme alone have no effect on susceptibility because quinolone interaction with the more sensitive enzyme causes cell death regardless of the drug affinity status of the less sensitive enzyme. The primary target of a
particular quinolone in a particular species then is determined by which enzyme is more sensitive to that quinolone. Patterns have emerged indicating that for current quinolones for most species of gram-negative bacteria DNA gyrase is the primary drug target and for many species of gram-positive bacteria topoisomerase IV is the primary target (21). Exceptions do occur, however, in that the primary target of sparflxacin in *Streptococcus pneumoniae* is DNA gyrase, indicating that relative targets are determined by drug structure (22).

Differences in quinolone sensitivity between DNA gyrase and topoisomerase IV have implications for risks of resistance development. With the occurrence of a resistance mutation in the more sensitive target enzyme, the level of susceptibility of this first-step mutant is determined either by the degree of alteration in primary target enzyme sensitivity determined by the particular mutation or the intrinsic level of resistance of the secondary target, whichever is less. Thus, the closer the levels of quinolone sensitivity of the two enzymes are to each other, the lower the increase in resistance that can occur with a first-step mutation in the primary target enzyme. This principle implies that the drug concentration above which two mutations will be required to select resistance will decrease as the level of concordance of sensitivity of the two enzymes increases. Furthermore, the extrapolation of this principle implies that drugs with potent and equal activity against both enzymes will have exceptionally low levels of resistance related to altered enzyme targets, since mutations in the genes of both enzyme targets must occur concurrently for initial resistance by target modification to occur.

With many current quinolones, however, there appear to be sufficient differences in sensitivity of the two target enzymes in many species for stepwise resistance to be selected. In which case highly resistant isolates can be selected sequentially with increasing or repeated quinolone exposure, resulting in accumulating mutations in *gyrA* and *parC*; in the most resistant isolates mutations have numbered two or more in each gene (23).

**Alterations in quinolone permeation**

**Alterations in the outer membrane and efflux systems.** In gram-positive bacteria, quinolones must traverse the cytoplasmic membrane, and in gram-negative bacteria they must traverse both the cytoplasmic membrane and the outer membrane to reach their topoisomerase targets. For many quinolones their size and zwitterionic charge configuration enhance their ability to diffuse across porin channels in the outer membrane (24). Reductions in porins have been associated with quinolone resistance, but more detailed studies have suggested that seldom is the level of reduction in diffusion by porin change alone sufficient to account for resistance and reductions in steady-state drug accumulation in growing bacteria (25). Increasingly recognized has been the common occurrence of endogenous efflux systems in many species of bacteria (26). These efflux systems are composed of a protein pump present in the cytoplasmic membrane either alone in gram-positive bacteria or linked to other proteins that span the periplasm and outer membrane in gram-negative bacteria. The pumps that have been shown to affect quinolone susceptibility...
belong to the major facilitator class of multidrug (MDR) pumps in gram-positive bacteria and the RND class in gram-negative bacteria (27). These MDR pumps are energized by proton motive force across the membrane and have broad substrate profiles. Intrinsic resistance in *Pseudomonas aeruginosa* and resistance in Mar mutants of *E. coli* associated with reductions in porin channels have been shown to dependent on intact MDR pumps such as the MexAB-OprM system in *P. aeruginosa* and the AcrAB-ToIC system in *E. coli* (28,29). In gram-positive bacteria, mutations causing hyperexpression of the NorA pump of *S. aureus* cause low-level resistance to some quinolones (30).

Quinolones appear to differ in the extent to which they are substrates for certain efflux pumps. In the case of NorA of *S. aureus* in particular, quinolones with greater hydrophobicity and other properties are less affected by hyperexpression of NorA (31). Similar correlations have been made for the several efflux systems identified in *P. aeruginosa* (32). The normal physiologic functions of these MDR pumps are not yet certain, but they are thought generally to function to remove toxins from the cell (33). Because quinolones are synthetic antimicrobials, they presumably played no direct role in the evolution of MDR pumps in Nature but are accidental substrates.

**Contribution of efflux pumps to quinolone resistance.** It is unclear the extent to which NorA-hyperexpressing mutants of *S. aureus* or Mar mutants of *E. coli* contribute to quinolone resistance in clinical isolates (34). But Mar mutants exhibit pleiotropic resistance (including tetracycline and chloramphenicol) and can be readily selected with tetracycline in the laboratory (35). In many cases in vitro, initial selections for quinolone resistance result in mutations in topoisomerase genes, but with *P. aeruginosa* pleiotropic mutants likely due to altered permeation are readily selected (36). In gram-positive bacteria, regulation of expression of some MDR pumps has been demonstrated. Mutants with increased expression of NorA induced by exposure to the quinolone norfloxacin have been described, implying regulation of expression (37). The factors that normally regulate the expression of these pumps, however, are unclear. It remains possible that the role of various MDR pumps in quinolone resistance may be more insidious, with physiologic increased expression under certain conditions of growth in vivo that results in reductions in the quinolone activity that would not be apparent by usual testing in vitro. Since the frequency of selection of resistant mutants decreases with the increasing ratio of quinolone concentration to MIC, physiologic increases in MIC in vivo due to increased MDR pump expression might contribute to higher frequencies of resistance selection in vivo (38).

**Transmissability of quinolone resistance**

The mechanisms of quinolone resistance described above all result from chromosomal mutations and not from acquired genes carried on plasmids. In merodiploid strains constructed in the laboratory quinolone-resistance alleles of gyr*A*, *gyr*B, *par*C, and *par*E are generally either recessive or codominant to their wildtype (susceptible) counterparts, but hyperexpression of resistance alleles on plasmids may confer some level of resistance (17,18,39). The
norA gene cloned on a plasmid in the laboratory can also confer quinolone resistance (31). Hyperexpression of any of these genes, however, may be toxic to the cell, and plasmid-mediated resistance by any of these mechanisms has not been described in clinical isolates. Recently, however, plasmid-mediated quinolone resistance was described for the first time in clinical isolates of Klebsiella pneumoniae (40). Resistance was also expressed upon transfer to E. coli in the laboratory. The mechanism of this resistance is not yet known nor is it clear the extent to which this type of resistance occurs in clinical resistant isolates.

Thus, in most cases a high prevalence of quinolone resistance appears to represent either selection of resistance due to chromosomal mutations in endogenous flora associated with intense quinolone exposure or spread of resistant strains, rather than spread of plasmids. In clinical isolates, the occurrence of high levels of resistance caused by multiple mutations implies the opportunity for repetitive drug exposures and the presence of reservoirs for organisms in which strains with the initial mutations may persist and be the source for selection of increamentally resistant strains with additional mutations upon further exposure to quinolones.

References


2 Susceptibility Testing of Quinolones and Links Between In Vitro and In Vivo Resistance

CJ Soussy

Introduction

The emergence of quinolone resistance during treatment depends on the site of the infection, the clinical situation and the bacterial species. It occurs with greatest frequency in infections caused by *P. aeruginosa*, *S. aureus* and bacteria for which the therapeutic ratio is low. It is the reason why one of the aims of the antibiotic susceptibility testing for quinolones is to detect resistant strains and strains weakly susceptible with minimal inhibitory concentrations close to the low breakpoint. This situation can be explained by the presence of a mechanism of resistance. Indeed, in some cases, the resistance is at a low level and may constitute the first step for selection of a higher level of resistance. So, it is important to analyze the phenotype of such strains by testing different compounds in order to modify the standard therapeutic regimens and obtain a high therapeutic indice. So, the emergence of resistance could be minimized and the clinical utility of these agents could be preserved.

I. In vitro susceptibility testing of quinolones

Clinical categorization, the classification of bacterial strains into susceptible, intermediate and resistant categories with regard to an antibiotic, is based on the critical values determined for minimum inhibitory concentrations (MICs) or for inhibition zone diameters. The critical values are recommended by national committees such as the Comité de l’Antibiogramme of the Société Française de Microbiologie (CASFM) in France, the British Society for Antimicrobial Chemotherapy (BSAC) in the United Kingdom, or the National Committee for Clinical Laboratory Standards (NCCLS) in the USA (1,2,3).

The MIC breakpoints are usually established on the basis of bacteriologic, pharmacokinetic and clinical criteria, as follows:

- MIC distribution of bacterial populations belonging to different species and harboring genetically and biochemically characterized resistance mechanisms;
- pharmacokinetics, at usual and maximum dosages, using the different routes of administration;
- correlation between the clinical and bacteriologic results for the therapeutic indications assigned by the different ministries of health.
The corresponding zone diameters breakpoints can be deduced from the MIC breakpoints by calculation of a regression line between the MIC values (logarithm to the base 2) and the inhibition diameters (arithmetic scale).

The first quinolones are approved for use in the treatment of urinary tract infections. According to the results of in vitro and in vivo activity, MIC breakpoints recommended by the CASFM for the first quinolones go from 2 and 4 for oxolinic acid to 16 and 32 for piromidic acid. However, for sensitivity testing in the routine laboratory, only one compound, generally nalidixic acid, may be used in most countries to predict the susceptibility to other first quinolones (Table 1). Indeed, the other compounds don’t cure the majority of the nalidixic acid resistant strains.

Among the currently available fluoroquinolones, norfloxacin, enoxacin and lomefloxicin are approved for use in the therapy of complicated and uncomplicated urinary tract infections. Pefloxacin, ofloxacin and ciprofloxacine extend the clinical applicability to additional sites and kinds of infections; sparfloxacine is specifically indicated in respiratory tract infections.

For all these fluoroquinolones, the low MIC breakpoint proposed in France when testing non fastidious organisms is 1 μg/ml while there is some variations in the high MIC breakpoint. The values proposed by the BSAC (2) are identical for ciprofloxacine but the low breakpoint is higher for ofloxacin (≤2). The values proposed by the NCCLS (3) are identical for ciprofloxacine but the low breakpoint is higher for enoxacin, lomefloxicin and ofloxacin (≤2) and for norfloxicin (≤4).

In staphylococci, the activity of all the currently available fluoroquinolones is similar. So, results obtained with one compound applies to the others. In Gram negative bacilli, ofloxacin and ciprofloxacine are respectively two fold and eight fold more active than pefloxacin. So, the strains susceptible to pefloxacin are susceptible to the other compounds. MICs of the fluoroquinolones slightly increase in strains resistant to nalidixic acid, but these strains remain susceptible in vitro (Table 2). In strains intermediate to pefloxacin, the MICs of ofloxacin and ciprofloxacine are at least ten fold higher than those observed in a susceptible strain, but can remain <1μg/ml. However, the therapeutic indice (drug level/MIC ratio) is lower (<8) and the risk of emergence of a resistant mutant highly increase (Table 3) (4).

Other compounds will be used in the near future: levofloxacine, trovafloxacin, grepafloxacine, moxifloxacine.
Table 1  Zone size and MIC breakpoints recommended by the CA SFM for non fastidious organisms

<table>
<thead>
<tr>
<th>Drug</th>
<th>Zone Size</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxolinic acid 1,2</td>
<td>≤ 2</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Flumequine 1,2</td>
<td>≤ 4</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>Nalidixic acid 1,2</td>
<td>≤ 8</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>Pipemidic acid 1,2</td>
<td>≤ 8</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>Piromidic acid 1,2</td>
<td>≤ 16</td>
<td>&gt; 32</td>
</tr>
<tr>
<td>Norfloxacin 1,3</td>
<td>≤ 1</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Lomefloxacin 1,3</td>
<td>≤ 1</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Enoxacin 1,3</td>
<td>≤ 1</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Pefloxacin 4</td>
<td>≤ 1</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Ofloxacin 4</td>
<td>≤ 1</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>Ciprofloxacin 4</td>
<td>≤ 1</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Sparfloxacin 4</td>
<td>≤ 1</td>
<td>&gt; 2</td>
</tr>
</tbody>
</table>

1. Result applies only to organisms isolated from urinary tract infections.
2. Result obtained with one compound applies to all first-generation quinolones.
3. There is cross-resistance to fluoroquinolones used in urinary tract infections, but the level of expression may vary depending on the bacterium.
4. There is cross-resistance to fluoroquinolones (FQ) used in systemic infections but the level of expression may vary depending on the bacterium:
   - Staphylococci: results obtained with one drug apply to others.
   - Streptococci (except S. pneumoniae) and enterococci: FQ are not advised.
   - S. pneumoniae: sparflaxacin, ofloxacin and ciprofloxacin can be used in pneumococcal infections, according to their therapeutic indications. Therefore, these compounds should be tested on this species. Enterobacteriaceae and Pseudomonas aeruginosa: every compound should be tested separately.

Table 2  Susceptibility to ofloxacin (OFX) and ciprofloxacin (CFX) according to resistance to nalidixic acid (NAL) and pefloxacin (PFX).

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NAL</td>
</tr>
<tr>
<td>E. coli NAL PFX S</td>
<td>4</td>
</tr>
<tr>
<td>E. coli NAL PFX S</td>
<td>128</td>
</tr>
<tr>
<td>E. coli NAL PFX I</td>
<td>512</td>
</tr>
</tbody>
</table>

S: susceptible I: intermediate R: resistant

Table 3  Therapeutic indice Cmax/MIC

<table>
<thead>
<tr>
<th>Strain</th>
<th>Therapeutic indice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax/MIC</td>
</tr>
<tr>
<td></td>
<td>PFX</td>
</tr>
<tr>
<td>E. coli NAL S PFX S</td>
<td>80</td>
</tr>
<tr>
<td>E. coli NAL R PFX S</td>
<td>20</td>
</tr>
<tr>
<td>E. coli NAL R PFX I</td>
<td>2.5</td>
</tr>
</tbody>
</table>
II. Evolution and present status of quinolone resistance

According to the French breakpoints, the frequency of resistance to quinolones in the main species encountered in human pathology is indicated Figure 1. The evolution of resistance is as follows during the last twenty-eight years in our hospital taken as an example:

In Enterobacteriaceae (Figure 2), the incidence of resistance to nalidixic acid, including the resistant and intermediate strains, slightly increased from 1969 to 1981, then stabilized between 15 and 20% until 1977; it never exceeded 20% of the strains during this 28 year period. The incidence of resistance to ciprofloxacin gradually increased from 1988 to 1993, then subsequently decreased; recently, an increase was observed again leading to the present situation at 16% (9% of resistant and 7% of intermediate strains) in our institution; this was probably explained by the increase of nalidixic acid high level resistant strains, especially in *E. coli*, harboring two mutations in the *gyrA* gene and one mutation in the *parC* gene; these strains generally appeared intermediate to ciprofloxacin (5).

The resistant rates for the different species are varying from 3% in *M. morganii* to 86% in *P. stuartii* (Figure 3 and 4). These results appear similar to those observed by Goldstein at the Saint Joseph Hospital in Paris (6).

![Figure 1 Resistance to nalidixic acid (N) and ciprofloxacin (C), Henri Mondor Hospital –1997](image-url)
Figure 2 Evolution of resistance to nalidixic acid and ciprofloxacin in enterobacteriaceae, Henri Mondor Hospital 1969-1997

![Graph showing percentage of resistance over years]

Figure 3 Resistance to nalidixic acid (N) and ciprofloxacin (C), Henri Mondor Hospital – 1997

![Bar chart showing resistance percentages for different bacteria]
In *P. aeruginosa* (Figure 5) naturally resistant to nalidixic acid, the incidence of resistance to ciprofloxacin increased from 1988 to 1993 and stabilized between 1993 and 1997 at approximately 37%; it never exceeded 40% of the strains.

In *A. baumannii* (Figure 5) the incidence of resistance to nalidixic acid, initially low, increased to 40% in the early 80s, 60% in 1985, 85% in 1990, leading to the present situation at 91%. The incidence of resistance to ciprofloxacin, initially high in 1992 decreased to 80% in 1993 and stabilized between 80 and 85% until 1997.

The incidence to resistance to fluoroquinolones in *S. aureus* (Figure 6), initially equal to 18% in 1985, subsequently increased, especially in MRSA from 1986 to 1993 then stabilized from 1993 leading to the present situation at 40%.

The increase of resistance to fluoroquinolones observed in *P. aeruginosa* and *S. aureus* in our hospital could be explained at least in part by the increase in the consumption of fluoroquinolones from 1986 to 1994 (2328 to 13249 g per year) (Figure 7). The stabilization in the resistance rates observed from 1993 corresponds to a decrease in the consumption of these antibiotics from 1994 to 1997 (13249 to 7512 g per year). The same situation was observed at the Claude Bernard Hospital in Paris during and after several years of extensive use of pefloxacin in an intensive care unit (7).
Figure 5: Evolution of resistance to nalidixic acid and ciprofloxacin, Henri Mondor Hospital 1969-1997

Figure 6: Evolution of Fluoroquinolone resistance in *S. aureus* strain, Henri Mondor Hospital 1995-1997

Figure 7: Evolution of fluoroquinolone usage (kg/y), Henri Mondor Hospital 1986-1997

Use of Quinolones in Food Animals and Potential Impact on Human Health
III. Relation between *in vitro* and *in vivo* resistance to quinolones

The observations that resistant bacterial isolates can occur in the setting of fluoroquinolone therapy are not unexpected (8). In the following cases, the emergence of resistance to quinolones could be probably explained by the weak susceptibility of the bacteria to these antibiotics, the clinical situation and the importance of consumption of these compounds.

1. Fluoroquinolones have been effective for treatment of urinary tract infections caused by *E. coli*. Resistant mutants were selected uncommonly during treatment. Indeed, the quinolone concentration is generally at least eightfold greater than the MIC in the urine during the treatment of urinary tract infection. However, in case of failure, no relationship was established between the isolation of the resistant strains and treatment with quinolones.

We reported the observation of acquisition of sequential *gyrA* mutations in a strain of *E. coli* isolated in the same patient during treatment of an urinary tract infection with quinolones (9):

*E. coli* HM72 was isolated from the urine of a 81 year old patient, during treatment with pipemidic acid 400 mg bid. This strain was resistant to nalidixic acid (MIC 128 µg/ml) but susceptible to norflaxacin, pefloxacin, ofloxacin, and ciprofloxacin (MICs 0.25, 0.5 and 0.08 mg/L respectively, four to eight fold superior to those observed for the susceptible reference strain KL16). Two days later, pipemidic acid was replaced by norflaxacin 400 mg bid, because of the results of susceptibility tests indicating resistance to pipemidic acid and susceptibility to norflaxacin. Ten days later, when the patient again developed fever and urinary tract symptoms, *E. coli* HM73, with MICs eight fold greater than those observed for HM72 (>256, 2, 4 and 0.5 µg/ml), was isolated from an urine specimen.

HM 72 and HM73 were indistinguishable according to the biotype and the plasmid content. Electrophoretic patterns of outer membrane proteins were identical. Little or no difference in accumulation of quinolones was found between the two strains.

The quinolones sensitivity of DNA gyrase for HM73 was 2 to 6 fold less than that observed for HM72 and 20 to 160 fold less than those observed for KL16. This ratio was close to that observed between the MICs, suggesting that the difference in resistance is due largely or solely to a difference in sensitivity of DNA gyrase to quinolone.

This suggestion was also supported by the results of the complementation tests of HM72 and HM73 with plasmid pJSW101 *gyr A*+ resulting in a decrease in MIC of ≥ 32 fold for ciprofloxacin. The DNA sequence of a fragment of *gyrA*, encompassing the quinolone resistance determining region, revealed in HM73 only two deduced amino acid changes Ser83 to Leu, presumably inherited from HM72, and Asp87 to Gly.

Because of the apparent genetic relationship of HM72 and HM73 and the correlation of the increments in MIC and resistance of DNA gyrase when the two strains
were compared. It is likely that HM73 was derived from HM72 by acquiring an additional mutation in the gyrA gene.

This observation emphasizes the potential risk of selection of a more resistant mutant in patients treated with usual doses of fluoroquinolones for infections caused by members of the enterobacteriaceae susceptible to fluoroquinolones but resistant to nalidixic and pipemidic acids.

2. Fluoroquinolones are widely used to treat patients with Salmonella infections but there are concerns about the emergence of quinolone resistance in this genus. For example, in Great Britain, the number of quinolone-resistant human isolates of S. typhimurium was increased from 0 to 14% between 1993 and 1996 (10,11) while only 0.5% of 4008 isolates of Salmonella were resistant in 1994 1995 in the United States (12). There have been several reports on the emergence of fluoroquinolone resistant clinical isolates of S. typhimurium during ciprofloxacin therapy (13,14). We isolated three quinolone resistant Salmonella, two of them during or after quinolone therapy (15).

- S. typhimurium AlhS (MIC 4, 0.12 and 0.02 µg/ml for nalidixic acid, norfloxacin and ciprofloxacin respectively) was isolated from the urine of a 49 year old AIDS patient who developed an urinary tract infection. He was treated with norfloxacin, 400 mg bid for 2 weeks, and the symptoms eventually improved. However, 1 month later, he relapsed and once again a fluoroquinolone sensitive S. typhimurium isolate was cultured from the urine. He was then given a 3 week course of norfloxacin and his symptoms resolved. But 2 months later, a second relapse occurred. Prostatic ultrasound examination showed signs compatible with a chronic prostatitis. He was then treated with 8 weeks of norfloxacin to be followed by a long term therapy with low doses of the same drug. During the course of this treatment, he relapsed again and a quinolone resistant S. typhimurium isolate, AlhR (MIC 256, 0.5 and 0.25), was obtained from urine.

- S. enteritidis OulS (MIC 8, 0.12 and 0.06) was isolated from the urine of a 40 year old renal transplant patient with acute pyelonephritis. The patient was treated with cefotaxime and his symptoms resolved. However, asymptomatic chronic carriage in the urinary tract persisted despite numerous treatments, including an 8 week course of therapy with trimethoprim sulfamethoxazole. After treatment with usual doses nalidixic acid, a fluoroquinolone resistant S. enteritidis OulR (MIC 256, 4 and 1) was cultured from the urine.

- S. hadar GueR (MIC 256, 2 and 0.5) was isolated from the stool of a 30 year old man who was admitted for diarrhea and low grade fever. The patient did not received antimicrobial therapy and his symptoms resolved spontaneously.

The strains OulS and OulR isolated from the same patient before and during quinolone therapy, respectively, exhibited identical patterns when compared by pulsed field gel electrophoresis. Identical S/F1I patterns were also observed for isolates AlhS and AlhR. This suggested that isolates AlhR and OulR were in vivo mutants selected during quinolone therapy.
The two cases with urinary tract infections were difficult to treat, with frequent relapses occurring even after prolonged therapy. In addition, treatment with quinolones seems to be associated with a high risk of selection of quinolone-resistant mutants; prolonged combination therapy might be proposed for the treatment of such infections. The last case, regarding gastroenteritis due to a fluoroquinolone-resistant isolate of Salmonella, shows that resistant Salmonella can be isolated even without prior fluoroquinolone exposure, especially in developing countries. The development of resistance to quinolones in Salmonella may threaten the future of these drugs as empirical agents for traveler's diarrhea.

3. Campylobacters have been recognized as a cause of diarrhea in man. Of these species C. jejuni and C. coli are the most frequently isolated. The fluoroquinolones have been utilized in the treatment of diarrhea caused by these species of Campylobacters although they appear less susceptible than Enterobacteriaceae (ciprofloxacin MIC 0.25 0.5 μg/ml). An increasing number of fluoroquinolone-resistant strains of Campylobacter has been reported, probably due to the large-scale use of these antibiotics. Endtz et al. (16) observed a striking increase in the rate of resistance to ciprofloxacin in Campylobacter strains from 0% to 11% between 1982 and 1989. In the same year, Rautelin et al. (17) have noted very similar rates. High percentages of ciprofloxacin resistance have also been shown by Reina et al. (18) between 1980 and 1991 in C. jejuni and C. coli. More recently, Sanchez et al. (19) observed a high rate of resistance in 1992, 51.3% for C. jejuni and 25% for C. coli, whereas no resistant strain was detected in 1988. Gootz and Martin (20) have found that DNA gyrase from quinolone-resistant mutants selected from C. jejuni UA534 was 100-fold less sensitive than the wild-type enzyme to inhibition by quinolones. In the same way, Segreti et al. (21) showed that DNA gyrase purified from the post-treatment C. jejuni clinical isolate was 8.8 fold less susceptible to inhibition by ciprofloxacin than the enzyme from the paired pre-treatment susceptible isolate. In addition, they observed that ciprofloxacin accumulation was diminished in post-treatment isolates. Wang et al. (22) demonstrated that a Thr 86 to Ile change in GyrA protein is associated with high level resistance to ciprofloxacin in C. jejuni clinical isolates. Recently, Charvalos et al. (23) have found that the multidrug resistance including to the quinolones, was associated with active efflux of drug in C. jejuni.

We isolated six Campylobacter resistant strains, five from stool specimens and one from blood cultures:

Three isolates of C. jejuni were highly resistant to fluoroquinolones, with ciprofloxacin MICs of 32 μg/ml. Another isolate of C. jejuni was low level resistant with nalidixic acid and ciprofloxacin MICs of 64 and 4 μg/ml, respectively. The two strains of C. coli were also high level resistant to fluoroquinolones with nalidixic acid MICs 256 512 μg/ml and ciprofloxacin MICs 32 64 μg/ml. These MICs were 8.16 fold more than wild type (4 and 0.25 0.5) for the low level resistant strain and 32 64 fold for the high level resistant strains.
All the \textit{C. jejuni} isolates had substitutions at Thr 86. The same mutation in \textit{gyrA}, leading to Thr-Ile substitution at position 86, was found in three of the four isolates, that are resistant to high levels of ciprofloxacin (MICs $\geq 32$ $\mu$g/ml). Similarly the three clinical isolates studied by Wang (22) had a Thr 86 to Ile substitution. By contrast, the isolate resistant to low level of ciprofloxacin (MIC $=4$ $\mu$g/ml) had an alanine at position 86. The \textit{C. coli} isolates are resistant to high level of ciprofloxacin (MICs $\geq 32\mu$g/ml). One of them was characterised by a mutation at position 86 leading to Thr-Ile. The other strain exhibited a mutation at position 90 leading to Asp-Tyr substitution. This was also the case for one of three laboratory mutants studied by Wang et al. (22).

These different results suggested that resistance to quinolones in clinical isolates of \textit{C. jejuni} and \textit{C. coli} is increasing and could be attributed to the expanding use of fluoroquinolones in human and animal infections caused by species with a relatively low susceptibility to these compounds (24). This resistance is due at least in part to alteration in the DNA gyrase and the mutation at position 86 of the \textit{gyrA} gene seems to play a critical role.

**Conclusion**

The resistance to fluoroquinolones has increased for several years, especially in certain clinical situations or in infections caused by certain bacteria (25,26). For example, strains resistant to nalidixic acid are significantly less susceptible to fluoroquinolones than the nalidixic acid susceptible strains. However, most of these strains appeared susceptible to fluoro-quinolones, according to the breakpoints. Thus, there is cross resistance to fluoro-quinolones used in human infections but the level of expression may vary depending on the compound. Pefloxacin which is intrinsically less active than ciprofloxacin against Gram negative bacteria will better detect strains with low level of resistance to fluoroquinolones. In order to avoid selection of resistant strains, several recommendations have been suggested (26):

- use quinolones only in recommended indications
- recognize strain with low level of resistance to fluoroquinolones
- use optimal dosing regimens in order to obtain high therapeutic indices (infection site drug level/MIC ratio $\geq 8$)
- avoid underdosing and make certain that other concomitantly administered agents do not interfere with the absorption of orally administered compounds
- use combination drug therapy in difficult to treat infections or in infections with high bacterial inoculum
- develop surveillance of resistance to detect new mechanisms and to prevent the extension of resistant strains
- develop more potent compounds.

**References**


Since the development of fluoroquinolones and their release in the United States in the mid 1980s, there has been extensive clinical use of these agents in both inpatients and outpatients (1). Until recently there were five fluoroquinolones available in the United States, norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin, and enoxacin, and ciprofloxacin and ofloxacin have received the widest use. In the past two years, four new agents have been released in the United States, levofloxacin, sparfloxacin, grepafloxacin, and trovafloxacin and are anticipated to contribute to increased usage of the class as a whole. This brief review will focus on the major areas of indicated uses in the United States and will include commentary on the circumstances in which acquired fluoroquinolone resistance has occurred in association with human use. Data from formal monitoring of clinical usage for specific indications are not available in the United States.

**Urinary tract infections.** All approved fluoroquinolones except sparfloxacin and grepafloxacin are indicated for the treatment of urinary tract infections. Efficacy is high in uncomplicated cystitis in young women, but other agents such as trimethoprim-sulfamethoxazole or nitrofurantoin are preferred as more cost-effective first-choice therapy (2). When fluoroquinolones are chosen, a 3-day regimen has been shown to be sufficient. Single-dose treatment may also be effective, but infections due to *Staphylococcus saprophyticus* respond less well to single-dose therapy. In uncomplicated pyelonephritis, studies indicate high cure rates for 7- to 10-day courses of ofloxacin or norfloxacin (3). Complicated urinary tract infections occurring in patients with structural and functional abnormalities of the urinary tract are more often caused by more difficult to treat pathogens including *Pseudomonas aeruginosa*. Cure rates of 75-80% for *P. aeruginosa* infections have been reported, but recurrent infections are common in this group of patients. Failures have been associated with acquired fluoroquinolone resistance in 10-20%. Surprisingly, quinolone-resistant *E. coli* infections have become a particular problem in Spain, and resistance has been associated with prior use of fluoroquinolones, urinary tract abnormalities, and presence of a catheter (4).

Prostatitis is an indication for ofloxacin, ciprofloxacin, and trovafloxacin. Chronic infections generally require 4- to 6-week courses of therapy with eradication rates of 67-91% in open studies (5). *E. coli* infections have been best eradicated with poorer response rates with infections caused by *P. aeruginosa* and enterococci.
Sexually transmitted diseases. Many fluoroquinolones (ofloxacin, enoxacin, ciprofloxacin, grepafloxacin, trovafloxacin) have approval for use in treatment of gonococcal urethritis and cervicitis and single-dose therapy is usually highly effective (6,7). Gonococci with reduced susceptibility to fluoroquinolones, however, have been identified in certain parts of the United States and have been associated with therapeutic failure (8). Ofloxacin, grepafloxacin, and trovafloxacin are approved for treatment of chlamydial infections but must be given for 7 days to be effective. For pelvic inflammatory disease, which may result for mixed infections that include gonococci, chlamydia, enteric bacteria, and anaerobes, trovafloxacin is the only quinolone approved for use alone, but ciprofloxacin in combination with an agent active against anaerobes is also approved and effective (9).

Gastrointestinal and abdominal infections. For treatment of bacterial gastroenteritis, ciprofloxacin is the only fluoroquinolone with approval in the United States, although other agents have been shown to be effective. The duration of diarrhea in both campylobacter and salmonella gastroenteritis may be shortened by norfloxacin (10), but persistence of Campylobacter jejuni in the stool after ciprofloxacin treatment has been associated with acquisition of resistance (11) and treatment failure. Fecal carriage of salmonella has also been prolonged after treatment with ciprofloxacin (12), but bacterial resistance was not reported. Several quinolones are effective in reducing symptoms in shigellosis, and a single 1-gram dose of ciprofloxacin is effective except in infection caused by Shigella dysenteriae type 1 (13). For travelers to areas of risk for bacterial gastroenteritis, presumptive therapy at the onset of diarrhea with ciprofloxacin given as a single dose (750 mg) or for 3 days with or without loperamide is recommended rather than use of quinolones in prophylaxis (14). For enteric fever ciprofloxacin and ofloxacin have been clinically effective with resolution of fever within 5 days (15), and because of resistance to other antimicrobial agents these quinolones are considered the agents of choice for typhoid fever.

Use of quinolones for treatment of other abdominal infections has included small numbers of patients with biliary tract infection with good response rates to ciprofloxacin (16), and in a more recent trial a combination of ciprofloxacin and metronidazole was shown to be comparable to imipenem for treatment of complicated intra-abdominal infections largely related to disease of the colon, appendix, or small bowel (17). Trovafloxacin is also approved for this use, but data are not yet published. Treatment of peritonitis associated with chronic peritoneal dialysis with systemically administered quinolones has heretofore been limited by the susceptibility of the usual staphylococcal pathogens (18). Norfloxacin has been shown to be effective as prophylaxis of spontaneous bacterial prophylaxis in patients at high risk due to cirrhosis (19), but this prolonged usage in low doses was associated with the emergence of quinolone resistance (20).

Respiratory tract infections. Many fluoroquinolones have approval for treatment of respiratory tract infections [ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, and (bronchitis only) lomefloxacin]. For acute bacterial exacerbations of chronic
bronchitis and community-acquired pneumonia there has been concern about the potency of ofloxacin and ciprofloxacin against the most commonly identified bacterial pathogen, *Streptococcus pneumoniae*. Eradication of *S. pneumoniae* has in some studies been less than eradication of the *Haemophilus influenzae*, a more susceptible pathogen (21). Sparfloxacin, levofloxacin, grepafloxacin, and trovafloxacin have increased potency against *S. pneumoniae*, and comparative studies have documented the ability of each of these agents to eradicate pneumococcal respiratory infections, some associated with bacteremia (22-24). Quinolone resistance has not been identified as a problem in *H. influenzae* or *S. pneumoniae* as yet, but a small number of resistant pneumococcal isolates have been reported from the UK (25). The spectrum of activity of these four newest quinolones like that of ciprofloxacin and ofloxacin also covers atypical pneumonia pathogens such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* spp., and thus are being recommended and marketed for routine and empiric treatment of community-acquired pneumonia in the setting of rising penicillin and cephalosporin resistance in *S. pneumoniae* (26). There is, however, concern that extensive use of newer fluoroquinolones for community-acquired respiratory tract infections may promote increasing pneumococcal resistance to these agents, and a concern that their future application for pediatric uses may pose a particular risk because the reservoir of pneumococci resides in this younger population. The documented overuse of antibiotics for treatment of respiratory tract infections, particularly those of likely viral origin, may further exacerbate the potential for resistance (27).

Pneumonia acquired in the hospital usually in association with endotracheal intubation more commonly involves *S. aureus* and gram-negative bacilli. For this indication, ciprofloxacin in high dose has been shown to be comparable to imipenem (28). Responses, however, were less in the subgroups with infections due to *S. aureus* and *P. aeruginosa*, and these pathogens persisting in sputum often acquired quinolone resistance. Ciprofloxacin has also been widely used for treatment of respiratory exacerbations in patients with cystic fibrosis in whom *P. aeruginosa* is the most common respiratory pathogen (29). Clinical responses were comparable to other conventional regimens in patients with mild to moderate exacerbations, but repetitive use was associated with rising resistance in *P. aeruginosa* isolated from sputum.

For mycobacterial infections, ciprofloxacin and ofloxacin have been used as second-line agents, particularly when needed for multidrug resistant strains. In comparative trials of multidrug regimens for pulmonary tuberculosis caused by susceptible strains of *M. tuberculosis*, rifampin appeared to be superior to ciprofloxacin, and ofloxacin appeared comparable to ethambutol (30,31). Ciprofloxacin has been used in multidrug regimens that are active in AIDS patients with disseminated *M. avium-intracellularare* infection, but these regimens were inferior to clarithromycin-containing regimens (32).

**Bone and joint infections.** Ciprofloxacin is approved for treatment of bone and joint infections in the United States. In small comparative trials, ciprofloxacin and ofloxacin have produced similar results to broad-spectrum cephalosporins or combinations
of gentamicin and penicillins (33-35). Most studies have been dominated by patients with mixed infections that include enteric gram-negative bacilli with overall provisional cure rates of about 75%, but patients with S. aureus and P. aeruginosa infections have responded similarly to the group as a whole. Infections associated with joint prostheses are difficult to cure without joint removal, but combinations of ofloxacin and rifampin given for 6-9 months have apparently cured as many as a half to two-thirds of patients with staphylococcal infections without joint removal (36). Failures have been associated with acquisition of resistance.

**Skin and skin-structure infections.** Ciprofloxacin, ofloxacin, levofloxacin, and trovafloxacin are all approved for this indication. Ciprofloxacin has been shown to be comparable to cefotaxime for cellulitis, wound infections, and infected skin ulcers, when mixed infections dominated gram-negative bacilli were the principal pathogens (37). Selection of quinolone resistance has been particularly problematic in this setting, and has been reported in a high proportion of staphylococci from diabetic patients treated with ciprofloxacin (38). For infections of the diabetic foot, additional coverage for anaerobic and gram-positive bacteria may be needed. Levofloxacin appears to have adequate coverage of gram-positive cocci for skin and soft-tissue infections (39), and trovafloxacin with its broad activity including gram-positive cocci and anaerobes is only fluoroquinolone approved as single agent therapy of diabetic foot infections; published data are not yet available, however.

**Systemic infections.** In patients with fever and neutropenia, ciprofloxacin or ofloxacin alone may not be adequate for those patients who are most severely ill (40), but ofloxacin has been used safely in the outpatient management of carefully selected low-risk patients (41). Choice of a quinolone would not be appropriate for therapy if a quinolone had been used for prophylaxis because of problems with resistant E. coli bacteremias which have been seen at some centers in which patients routinely received quinolone prophylaxis during episodes of neutropenia (42-44). Ciprofloxacin in combination with rifampin has been used for treatment of patients with S. aureus right-sided endocarditis (45,46). Quinolones or third-generation cephalosporins are now commonly used in many U.S. hospitals in place of aminoglycosides for gram-negative coverage in systemically ill patients in intensive care units. In some hospitals, there has been an increase in resistance to quinolones since their introduction, with the most commonly affected organisms being S. aureus, usually methicillin-resistant strains (MRSA), and P. aeruginosa (47).

**Uses in prophylaxis or eradication of colonization.** In a number of studies use of quinolones as prophylaxis in patients with neutropenia has been effective in reducing the occurrence of gram-negative bacteremia, but in bone marrow transplant recipients their use has also been associated with an increased incidence of viridans streptococcal bacteremia (40). In some cancer centers prophylactic use of quinolones has been associated with an increasing incidence of quinolone-resistant E. coli bacteremias (42-44). The organisms involved appear to be largely distinct strain types and to have
multiple resistance mutations, suggesting sequential selection of endogenous flora. Eradication of nasopharyngeal colonization with *Neisseria meningitidis* has been accomplished by single doses of ciprofloxacin and ofloxacin (48), but attempts at eradication of nasal and skin carriage of MRSA have been largely unsuccessful and associated with selection of resistance (49). Secretion of fluoroquinolones in sweat may contribute to selection of resistance among staphylococci colonizing the skin (50). Only trovafloxacin has been approved for prophylaxis in colorectal and pelvic surgery in the United States. Data on this use are not yet published.

**Epidemiologic features of quinolone resistance associated with human quinolone use.** In many studies the single strongest risk factor for acquisition of quinolone resistance has been use of quinolones for either therapy or prophylaxis in individual patients, and in some studies there has been a dose-response relationship with increasing amounts of drug exposure correlating with increasing risk (42,51,52). Opportunity for spread of resistant organisms may also contribute in some cases, such as with infections caused by gonococci with reduced quinolone susceptibility that were shown to be less likely to be seen on gram staining of urethral specimens than fully susceptible isolates and thus may be more likely to go undetected and possibly untreated (8). Also for nosocomial pathogens such as *S. aureus*, which may be multidrug resistant, two additional factors may amplify quinolone resistance. First, these strains may be spread from patient to patient in the hospital environment, as supported by the observation that over time after the introduction of use ciprofloxacin in one hospital an increasing proportion of patients with ciprofloxacin-resistant MRSA had not received a ciprofloxacin and thus would not have selected a resistant strain from their own endogenous flora (53,54). Second, once ciprofloxacin resistance is acquired by an already multiply resistant strain of staphylococci, this strain may be easily amplified or selected by patient exposure to any of several antibiotics (57). In vitro studies and studies simulating drug concentration-time profiles in serum suggest that exposure of susceptible bacteria to peak drug concentrations in excess of 10-fold the MIC reduces the likelihood of selection of resistant subpopulations (55,56), a finding that is likely related to the observation that single spontaneously occurring mutations usually cause an increment of resistance of 10-fold or less for current fluoroquinolones (57). Thus, such mutants in a population may still be killed by drug concentrations in excess of this amount. This principle would imply that use of high doses of fluoroquinolones for brief periods will carry a lower risk of resistance than lower doses used over prolonged periods. Inverse correlations have been made between the ratio of AUC (which is related to dose) of ciprofloxacin and MIC of the infecting pathogen and development of resistance in patients undergoing treatment of lower respiratory tract infections (58).

**Summary.** The newer quinolones have been widely and in many cases effectively used in human medicine. Associated with their use (and perhaps overuse) has been the emergence of resistance in some organisms, *P. aeruginosa* and MRSA in particular. Resistance acquired during therapy has also
occurred with campylobacters causing gastroenteritis. Resistance due to multiple mutations developing in initially highly susceptible pathogens such as E. coli and gonococci was initially surprising and may have resulted from intense selection pressures, reservoirs for persistence of organisms with intermediate levels of resistance, and person-to-person spread. As expanded indications for treatment of community-acquired respiratory tract infections are developed with the newest quinolones with enhanced potency against pneumococci, (including penicillin-resistant strains), the challenge will be to guide the appropriate targeting of their usage to minimize the risks of development of resistance in pneumococci.

References


4 REVIEW OF THE CLINICAL USE OF QUINOLONES IN HUMAN MEDICINE: EASTERN HEMISPHERE

Sin-Yew Wong

Introduction

The introduction of fluoroquinolone agents into clinical practice is an important advance in the therapeutic armamentarium against infections (1,2). These agents are considered by many as the most important new class of antimicrobial agents in the 1980s and 1990s.

The initial quinolones and their related naphthyridines have been known since the early 1960s. Some of these compounds include nalidixic acid and cinoxacin which suffered from numerous drawbacks including limited spectrum of activity (primarily against Enterobacteriaceae), toxicity (especially affecting the central nervous system and gastrointestinal tract) and rapid emergence of resistance during therapy. Their use was often limited to treatment of urinary tract and some gastrointestinal infections.

Chemical modifications of the quinolone nucleus (including fluorine atom at position C-6 and piperazine ring or substituted piperazine derivative at position C-7) resulted in marked improvement in potency, spectrum of activity and the reduction of high level resistance by a single step mutation. Many of these recent compounds including norfloxacin, ciprofloxacin, pefloxacin, ofloxacin, lomefloxacin, enoxacin, levofloxacin, fleroxacin, sparfloxacin have entered into clinical use with considerable success in the treatment of clinical infections associated with Enterobacteriaceae, Pseudomonas aeruginosa and with limited success in Gram positive infections. Further modifications have resulted in enhanced activity of these compounds against Gram positive organisms (substitution of a pyrrolidine derivative or azabicyclo side chain at position C-7) and anaerobes (various modifications including addition of difluorophenyl side chain at position C-1, addition of halogen atom at C-8). At least two of these new fluoroquinolones, trovafloxacin and grepafloxacin have been registered in the past year in the West and will likely receive registration for clinical use in Asia in the coming months.

This review will focus on the clinical use of fluoroquinolones in Asia reported in the English medical literature.
Clinical Use

The fluoroquinolones are now extensively used because of their wide spectrum of in vitro activity, good bioavailability low toxicity, good tissue distribution and penetration (2).

Sexually Transmitted Disease

Fluoroquinolones are an important class of antimicrobials in the treatment of sexually transmitted disease (3,4,5). For gonococcal infections, numerous trials in Asia have demonstrated that single doses of fluoroquinolones (ciprofloxacin 250-500mg, norfloxacin 800mg, ofloxacin 400mg) were highly effective in uncomplicated urethritis and cervicitis with cure rates of 95% (3-7). However, caution is recommended as decreased susceptibility to fluoroquinolones is emerging rapidly in Asia (HK and Thailand) (8-11). In Japan in 1992, N. gonorrhoea strains had MIC90 values for norfloxacin, ofloxacin and ciprofloxacin which were 8 fold higher than those against strains isolated in 81-84 (11). High level resistance to fluoroquinolones may be present in up to 15% in patients who fail an initial course of ofloxacin (9). The problem of fluoroquinolone resistant gonococci may become a major therapeutic problem in future and needs to be closely monitored (12,13).

For chancroid, the fluoroquinolones have also been highly effective with cure rates approaching 100% with single oral doses (ciprofloxacin 500mg, norfloxacin 800mg, ofloxacin 400mg) (3,4,14).

Ofloxacin 400mg bid for 7 days is effective in the treatment of NGU due to Chlamydia trachomatis but is not frequently used because of cost considerations. Doxycycline for 7 days or single dose azithromycin is preferred by many Asian centres (3).

Enteric Fever

In recent years, multidrug resistant strains of Salmonella typhi have appeared in numerous Asian countries, and in many areas, these now cause the majority of infections (15-18). Fluoroquinolones are highly effective in the treatment of typhoid and paratyphoid fever and are now considered by many as the oral agent of choice. Numerous clinical trials from Asia have been reported on their efficacy and effectiveness with the additional benefit of shorter duration of treatment. In adults, oral ciprofloxacin for 7 days (19,20), ofloxacin for 3-5 days (21,22) and fleroxacin (23) have been reported to be as effective as intravenous ceftriaxone (22,23). Fluoroquinolones have also been used in children with similar success (21,24). Fluoroquinolones were also effective in eliminating Salmonella typhi from the stools without the development of a carrier state (25).

Salmonella typhi isolates with reduced susceptibility or resistance to fluoroquinolones has recently emerged in Asia (26-31). In Vietnam, for S. typhi isolates with resistance to nalidixic acid but "susceptible" to ofloxacin based on current recommended breakpoint values, treatment with short course of ofloxacin is associated with higher failure rates (31).
Gastrointestinal Infections and Traveller’s Diarrhoea

The fluoroquinolones are not only highly active in vitro against a broad range of enteric pathogens but also possess many of the properties desirable for the treatment of these infections viz; intracellular activity, adequate luminal and tissue levels, good enterohepatic circulation and good oral bioavailability.

In many developing nations in Asia, the treatment of drug-resistant shigellosis has become a major public health challenge. A study of shigellosis in Thai children (32) revealed that 45% of cases were in children with the predominant species being *S. flexneri* (74%). A high rate of resistance to conventionally used antibiotics ampicillin and co-trimoxazole was present with only 17% and 22% sensitive respectively in 1993. The antimicrobial of choice for the treatment of shigellosis have been changed from ampicillin to TMP-SMZ and recently to fluoroquinolones. Another study from Thailand (33) reported that norfloxacin was effective for the eradication of *Shigella* species from the stools of both adults and children and that no short term adverse effects were encountered.

In China, a study of childhood diarrhoea in children reported that (34) the most frequently detected pathogen was ETEC (20%). Other bacterial species isolated included other *E. coli, Salmonella, Aeromonas, Shigella, Campylobacter* and *Vibrio* spp. All the isolates were susceptible in vitro to ciprofloxacin.

Fluoroquinolones are the current antimicrobials recommended for treatment and prophylaxis of traveller’s diarrhoea (TD) acquired from tropical countries including those in Asia. Ciprofloxacin, norfloxacin, norfloxacin and fleroxacin are efficacious in the treatment of TD. However, there is evidence to suggest increasing resistance to this class of drugs due to widespread and injudicious use. A study of antibiotic resistance among diarrhoeal pathogens isolated in Thailand over a period of 15 years found ciprofloxacin resistance in *Campylobacter* isolates increased from 0% before 1991 to 84% in 1995 (35). Another report from Murphy, (36) of US troops deployed to Thailand found *Campylobacter* species as the major cause of diarrhoea and >70% of the isolates were resistant to ciprofloxacin. Many of these isolates also demonstrated a concomitant resistance to azithromycin, an effective alternative antibiotic, making the clinical management of such cases increasingly difficult.

*Vibrio cholerae*, an important cause of diarrhoeal disease in this part of the world and *V. cholerae* 0139, an emerging pathogen remain susceptible to quinolones as well as tetracyclines. Khan found that single dose ciprofloxacin is effective in the treatment of diarrhoea caused by *V. cholera* 01 or 0139 in a cohort of Bangladeshi men with moderate to severe dehydration and is better than single dose doxycycline in the eradication of *V. cholerae* from stool (37). It may also be the preferred treatment in areas where tetracycline resistance is common.
Urinary Tract Infection

The pharmacokinetic properties of the fluoroquinolones after oral therapy result in high concentrations in the urine as well as the prostatic fluid and tissue (38,39). Clinical trials of short term (single dose and 3 day regimens) for uncomplicated lower urinary tract infections demonstrate that it is highly efficacious (38).

Although there was only one published study on the use of fluoroquinolones for urinary tract infections in Asia (40), it is likely that fluoroquinolones are commonly used to treat both uncomplicated and complicated urinary tract infections in tropical countries.

Outpatient Management of Febrile Neutropenia

Hospitalization and intravenous broad spectrum antibiotic therapy has traditionally been considered standard therapy for febrile neutropenic patients with cancer. In many developing countries, this is not always possible. Reports from Pakistan have shown that ofloxacin monotherapy was as effective as combination intravenous therapy in febrile neutropenic patients with a short duration of neutropenia (41). In a subsequent study, self administered ofloxacin in an outpatient setting was as effective as inpatient therapy for the management of febrile neutropenia in patients with non-haematologic malignancies (42,43). In the paediatric age group, 91% of the febrile episodes responded to outpatient ofloxacin therapy and only 9% required subsequent hospitalization (44).

Respiratory Tract Infection

Clinical trials of the early fluoroquinolones including ofloxacin, ciprofloxacin, enoxacin and sparflloxacin have demonstrated efficacy in community acquired pneumonia, acute exacerbation of chronic bronchitis and sinusitis (45-48). However, they have often not been used as first line therapeutic agents in these conditions because of reservations of their relatively modest activity against pneumococci. Several highly publicised cases of fluoroquinolone failures in patients with pneumococcal infection were major contributing factors that limited their use (49,50).

The new fluoroquinolones including grepafloxacin and trovafloxacin have enhanced activity against Gram positive organisms including penicillin resistant pneumococci will likely change the earlier reservations. Together with the in vitro activity against the “atypical” respiratory pathogens (Legionella, Chlamydia and Mycoplasma), these new fluoroquinolones have activity against the majority of community acquired bacterial respiratory pathogens and are likely to be aggressively marketed.

There was little published literature of fluoroquinolone use in respiratory tract infections in Asia. It is likely that β-lactams, macrolides and trimethoprim-sulfamethoxazole are more frequent choices because of cost considerations.

Drug-Resistant Tuberculosis

Tuberculosis remains an important cause of morbidity and mortality in Asia and the gravity of multidrug resistance (MDR) TB has caused great global concern. Ofloxacin
at a dose of 400-800mg/day is a frequently used 2nd line drug in MDR TB in Asia and has been reported to have sputum conversion rates of 59-79% (51-56).

**Paediatric Use**

Fluoroquinolone induced cartilage toxicity has been observed in experimental juvenile animal studies and led to reservations in their use in the paediatric patient population. Recent reviews have suggested that the adverse event pattern in children receiving fluoroquinolones was similar to that observed in adults (57) and that prolonged therapy was effective and well tolerated with out significant arthopathy (58). In Asia, fluoroquinolones have been used extensively in children with multidrug resistant typhoid with considerable success and safety (21,24).

**Bone and Joint Infections**

There are numerous studies that reported a favourable outcome of 65-95% of patients with osteomyelitis treated with fluoroquinolones (59-62). The major advantage was the use of oral fluoroquinolone therapy compared with parenteral therapy for other agents. Patients with Gram positive infection, especially *S. aureus* were associated with higher relapse and recurrence (60). Several small studies on the use of rifampicin plus quinolones in patients with infected prostheses due to Staph species was encouraging (63,64). There is also data to support the use of the fluoroquinolones (ciprofloxacin and ofloxacin) in the treatment of diabetic foot infections (65,66).

One study from Taiwan reported on the successful use of fluoroquinolones in the treatment of osteomyelitis and septic arthritis (67). Although there are no further published reports from Asia, it is likely that fluoroquinolones are used frequently in such infections.

**Other Infections**

The fluoroquinolones have good in vitro activity against *Rickettsia* spp and *Coxiella burnetii*. In anecdotal reports, ciprofloxacin has been used to treat patients with typhus in Asia (68). Because of cost considerations, doxycycline is the preferred agent.

In a small study from India, norfloxacin in combination with proton pump inhibitors were successful in eradicating *H. pylori* (69).

**Emergence of Fluoroquinolone Resistance**

In many Asian countries, there is wide spread availability and uncontrolled use of antimicrobials. Public health and education programmes are hampered by limited resources and finance. This coupled with a high endemicity rate for typhoid, gonorrhoea, provides a selective pressure on a large bacterial population to develop resistance to antimicrobials including the fluoroquinolones. There are factors which are unique to Asia that may have contributed to the development of resistance to antibiotics in general and these include:

1. Uncontrolled and unlicensed proprietary/“traditional” drugs containing varying amounts of antibiotics that can be bought over the counter
2. Lack of proper guidelines/programmes to control/restrict antibiotic usage both in and out of institutions

3. Lack of sufficient number of properly trained clinical pharmacologists and clinical Infectious Disease physicians to advise on usage and indications

4. Poor patient compliance due to poor insight coupled with high cost of certain antibiotics have led to suboptimal dosing and duration of treatment

5. Lax registration requirements and post-marketing surveillance as well as irresponsible pharmaceutical marketing leading to uncontrolled influx of antibiotics into the market without proper education on their uses.

Concluding Comments

Since the introduction of the fluoroquinolones into clinical practice, their efficacy, convenience and general low toxicity in the treatment of a wide spectrum of infections has resulted in their extensive use. They are a victim of their own success because with widespread use in humans and animal husbandry, the looming spectre of drug resistance especially in Neisseria, Salmonella and Campylobacter is worrisome.

The fluoroquinolones have made a major impact in our treatment of many infections but we need to focus their use in areas which there is benefit over conventional agents in efficacy, costs, safety or where there are limited therapeutic alternatives. Otherwise, the development of widespread resistance will severely limit the life span and use of these agents in our fight against infections.

References


51. A controlled study of rifabutin and an uncontrolled study of ofloxacin in the retreatment of patients with pulmonary tuberculosis resistant to isoniazid, streptomycin and rifampicin. Hong Kong Chest Service/British Medical Research Council. Tuber Lung Dis, 1992, Feb; 73(1):59-67


5 Quinolone Resistance in Community-Acquired Pathogens in South Africa

HJ Koornhof

Fluoroquinolones are widely used in South Africa. They are only available from chemists on prescription from medical practitioners or when prescribed in hospitals by attending physicians. All academic hospitals and most private and larger provincial hospitals have committees advising clinicians on the use of antimicrobial agents and in some hospitals the prescribing of key antibiotics is controlled. This applies to the use of quinolones in most academic hospitals where motivation followed by authorization by dedicated experts in the field of antimicrobial therapy were initially required. In recent years however, control of quinolone use in hospitals has been relaxed and in some academic hospital clinics they may be prescribed for urinary tract infections without special motivation. Doctors in private practice have a free hand in prescribing antibiotics and fluoroquinolones are freely used by them, often inappropriately, e.g., as empiric treatment for respiratory infections.

In this study, records of drug susceptibility testing performed by hospital-based laboratories that are run by the South African Institute for Medical Research countrywide were analysed. The period covered was from September 1997 to the end of March 1998. For comparison, the incidence figures of quinolone resistance encountered in academic hospitals, as well as in the private sector, were obtained and will feature in this presentation.

Methodologies:
Drug susceptibility testing was performed according to the Kirby-Bauer disc diffusion technique using NCCLS breakpoints in most laboratories although a few private and academic hospitals use breakpoint concentrations in agar media. Four rural laboratories in the Free State and Northern Cape provinces used the Stokes disc diffusion method (Table 2 and Table 4). In academic hospital laboratories and several private

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparative Usage of Fluoroquinolones in South Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agents</td>
<td>Units in millions p.a (% of group)</td>
</tr>
<tr>
<td>All</td>
<td>8.9 (100)</td>
</tr>
<tr>
<td>Oral</td>
<td>7.7 (87)</td>
</tr>
<tr>
<td>Oral Fl-quinis</td>
<td>0.6 (8)</td>
</tr>
<tr>
<td>Oral Cephs</td>
<td>1.5 (20)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>1.2 (13)</td>
</tr>
<tr>
<td>0.10 (9)</td>
<td>0.10 (9)</td>
</tr>
<tr>
<td>Par Cephs</td>
<td>0.92 (80)</td>
</tr>
</tbody>
</table>
laboratories, blood culture isolates are subjected to minimal inhibitory concentration determinations involving appropriate antimicrobial agents. Investigations on salmonellae and Shigella dysenteriae type 1 from KwaZulu Natal were conducted at the King Edward VIII Hospital under Professor W H Strüm's direction and the findings were kindly supplied by Dr Das Pillay.

**Community-acquired vs. hospital-acquired infections**

Isolates from urine specimens in the rural laboratories were considered to be a fair reflection of the etiology of community-acquired infections (Table 2 to Table 6). More than 95% of isolates from the academic hospitals (Table 9) were likely to have been hospital-acquired while between 10% and 20% of isolates from the urban private sector were community-acquired (Table 8 and Table 9).

**Results and discussion**

An indication of the comparative usage of antimicrobial agents in South Africa is given in Table 1. Approximately 80% of fluoroquinolones used is administered orally and by far the majority is prescribed for patients with community-acquired infections. Oral and injectable quinolones constitute approximately 10% of the total consumption of antibiotics used in the respective routes of administration.

Table 2 to Table 6 indicate quinolone resistance in isolates from the urine in rural regions in South Africa. Regional differences are striking and lowest incidence figures were encountered in the sparsely populated Free State and Northern Cape provinces. Although the numbers are small, it is especially noticeable in the case of *Staphylococcus aureus* isolates where 1.6% of isolates were resistant to quinolones in this

---

**Table 2** Quinolone resistance in urine samples from rural South Africa

<table>
<thead>
<tr>
<th></th>
<th>Percentage quinolone-resistant isolated in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>North Provs&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Total  R%</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2590  2.7</td>
</tr>
<tr>
<td><strong>Pseudomonas</strong></td>
<td>1477  1.1</td>
</tr>
<tr>
<td><strong>Acinetobacter</strong></td>
<td>138   6.5</td>
</tr>
<tr>
<td><strong>Staph. aureus</strong></td>
<td>100   28</td>
</tr>
<tr>
<td></td>
<td>104   30</td>
</tr>
</tbody>
</table>

(a) Some of the isolates, especially *S. aureus* and Acinetobacter strains, may have been endogenous contaminants

(b) Northern provinces

(c) Free State and Northern Cape

(d) Western Cape

(e) Western Cape, day hospital clinics only

(f) Dash (-) denotes not tested

---

70 Use of Quinolones in Food Animals and Potential Impact on Human Health
region as opposed to 30 percent in the northern provinces of South Africa which have a much higher population density. The fact that the laboratories in the Free State and Northern Cape provinces use the Stokes method of disc susceptibility testing is unlikely to compromise comparison with the findings of other laboratories using the Kirby-Bauer technique. The high percentage of quinolone resistance in *Pseudomonas* organisms detected by the Stokes method (Table 4), is in accordance with this assumption, indicating that quinolone resistance was unlikely to have been missed in these laboratories. As quinolones are not recommended for use in staphylococcal infections, quinolone susceptibility testing of these bacteria is not performed in many laboratories in South Africa. Also, in the case of urine samples, many of the *S. aureus* isolates are likely to be contaminants and for this reason were not subjected to susceptibility testing. Differences in quinolone resistance in *S. aureus* were encountered amongst isolates other than from urine samples in the rural provinces (Table 7) where northern provinces again show a high incidence compared with the incidence in a provincial urban hospital in the Eastern Cape Province. Most striking however is the low incidence of quinolone resistance (3.2%) in *S. aureus* at the Chris Hani Baragwanath Hospital in Johannesburg where quinolone use is restricted (see Table 9). Also, the population it serves comes predominantly from a poor socio-economic background where patients are unlikely to be exposed to the luxury of expensive quinolone treatment. The low incidence at the Chris Hani Baragwanath Hospital is in stark contrast with the 23% resistance encountered in *S. aureus* isolates from the private sector. Similar geographical differences in staphylococcal resistance to quinolones were seen in the private sector varying from 5% in Durban to 51% in Johannesburg.

### Table 3
Comparative resistance to quinolones in urine isolates from rural South Africa: Northern Provinces

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Number and percentage of isolates resistant to:</th>
<th>Quinolones</th>
<th>Gentsamicin</th>
<th>Cefazolin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>No (%)</td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>2590</td>
<td>69 (2.7)</td>
<td>413 (16)</td>
<td>928 (36)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1477</td>
<td>22 (1.5)</td>
<td>1350 (6.1)</td>
<td>1477 (10.8)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>138</td>
<td>9 (6.5)</td>
<td>25 (18)</td>
<td>-</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>100</td>
<td>28 (28)</td>
<td>40 (40)</td>
<td>-</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>104</td>
<td>31 (30)</td>
<td>51 (49)</td>
<td>45 (43)</td>
</tr>
</tbody>
</table>

(a) Dash (-) denotes not tested/irrelevant
The incidence of quinolone resistance in Enterobacteriaceae organisms, amongst which *Escherichia coli* and *Klebsiella* species predominate, is still relatively low in rural areas and vary from 5.4% in the Eastern Cape to less than 1% in the Free State and Northern Cape and in day hospital clinics in small Western Cape Towns.

Resistance rates of fluoroquinolones are higher among *Pseudomonas aeruginosa* isolates from urine samples compared with those of the Enterobacteriaceae in rural settings (Table 2) but are much higher in the private sector (Table 8). These high rates may be related to the nature of *Pseudomonas* infections that tend to be chronic as in urinary tract infections in paraplegics and respiratory infections in patients with cystic fibrosis. The chronicity of these infections requiring prolonged treatment creates an ideal situation for selection of resistant mutants. Also, a sharp increase in quinolone resistance has been experienced in hospitals in South Africa in recent years and this trend is illustrated in Table 9 where high resistance figures in the Johannesburg Hospital and private hospitals are shown as opposed to those at the Chris Hani Baragwanath Hospital.

Resistance to *Acinetobacter* isolates is very high in both rural and urban settings and several academic hospitals have been experiencing *Acinetobacter* outbreaks in their intensive care units.

As the majority of salmonella and shigella infections in South Africa are likely to be community acquired, antimicrobial resistance profiles in these bacteria were also analysed (Table 10). All salmonella and shigella isolates, including 695 non-typhoid salmonellae, 268 *Salmonella typhi* strains, 392 *Shigella dysenteriae* type 1 and 448 shigellae other than *S. dysenteriae* type 1

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Total</th>
<th>Quinolones</th>
<th>Gentamicin</th>
<th>Cefazolin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacteriaceae</em></td>
<td>1800</td>
<td>2 (0.1)</td>
<td>214 (4.8)</td>
<td>156 (9.5)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1073</td>
<td>1 (0.1)</td>
<td>40 (3.7)</td>
<td>76 (4.0)</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>23</td>
<td>3 (13)</td>
<td>3 (13)</td>
<td>-</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>9</td>
<td>0</td>
<td>4 (44)</td>
<td>-</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>64</td>
<td>1 (1.6)</td>
<td>11 (17)</td>
<td>-</td>
</tr>
</tbody>
</table>

(a) Total of 1648 isolates tested for cefazolin resistance
(b) Dash (-) denotes not tested or irrelevant
were susceptible to fluoroquinolones. Only 6 out of 687 shigella isolates (0.9%) tested for nalidixic acid resistance were resistant to this agent. All *S. typhi* isolates in South Africa were also found to be susceptible to ampicillin, chloramphenicol, cotrimoxazole and ceftriaxone while all *S. dysenteriae* type 1 strains were resistant to ampicillin and 99% to cotrimoxazole. Thirty-five percent of non-typhoid salmonellae outside KwaZulu Natal were found to be resistant to ampicillin and less than 2% to ceftriaxone. In KwaZulu Natal some *Salmonella typhi-murium* isolates were found to produce an extended spectrum beta-lactamase.

With regard to fluoroquinolone resistance in animals in South Africa, surveillance done by the Onderstepoort Veterinary Institute indicated that 5 out of 347 isolates (1.4%) were fully resistant, based on NCCLS disc diffusion technology and breakpoints. Enrofloxacin, danofloxacin and norfloxacin are licenced for veterinary use in South Africa by prescription only but are widely used both therapeutically and prophylactically, especially in poultry and pigs (Personal communication, Dr M. M. Henton, Onderstepoort Veterinary Institute).

In conclusion, it is clear that quinolone resistance has become a problem in South Africa, also in rural settings where the bulk of isolates recorded in this presentation come from community-acquired infections. The escalating incidence of quinolone resistance in *Pseudomonas* isolates and the patchy but alarmingly high incidence of resistance in *S. aureus* isolates in some hospitals and regions does not augur well for the future. Reassuring is the absence of fluoroquinolone resistance in salmonella and shigella isolates.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Total</th>
<th>Quinolones</th>
<th>Gentamicin</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>1875</td>
<td>32 (1.7)</td>
<td>98 (12)</td>
<td>28 (3.5)</td>
</tr>
<tr>
<td>E. coli</td>
<td>1248</td>
<td>20 (1.6)</td>
<td>11 (3.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>45</td>
<td>5 (11)</td>
<td>19 (42)</td>
<td>-</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>34</td>
<td>11 (32)</td>
<td>11 (32)</td>
<td>-</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(a) Number of isolates tested against gentamicin
(b) Number of isolates tested against ceftriaxone
(c) Dash (-) denotes not tested or irrelevant
### Table 6: Comparative resistance to quinolones in urine isolates from rural South Africa: Western Cape, day hospital clinics only

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Total</th>
<th>No (%)</th>
<th>No (%)</th>
<th>No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>235</td>
<td>2 (0.9)</td>
<td>2 (2.7)*</td>
<td>0*</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>190</td>
<td>2 (1.1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(a) Only 74 isolates tested for gentamicin and ceftriaxone resistance
(b) Dash (-) denotes not tested or irrelevant

### Table 7: Percentage quinolone resistance in isolates, excluding those from urine, in rural South Africa, September 1997 - March 1998

<table>
<thead>
<tr>
<th>Percentage quinolone-resistant isolates in:</th>
<th>Northern Prov*</th>
<th>Eastern Cape b</th>
<th>Western Cape c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td>No (%) R</td>
<td>No (%) R</td>
<td>No (%) R</td>
<td>No (%) R</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>5465 3.7</td>
<td>203 5.4</td>
<td>244 3.5</td>
<td>8111 4.7</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>625 2.9</td>
<td>52 1.9</td>
<td>339 3.5</td>
<td>1016 3.2</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>857 15.7</td>
<td>95 2.1</td>
<td>-</td>
<td>962 14.3</td>
</tr>
</tbody>
</table>

(a) Clinics in northern provinces
(b) Eastern Cape, one hospital clinic only
(c) Western Cape clinics
(d) Dash (-) denotes testing not performed
Table 8  Quinolone Resistance in The Private Sector in South Africa

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Johannesburg</th>
<th>Pretoria</th>
<th>Cape Town</th>
<th>Durban</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Resist (%)</td>
<td>Total</td>
<td>Resist (%)</td>
<td>Total</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1204</td>
<td>96 (8)</td>
<td>3826</td>
<td>77 (2)</td>
<td>700</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>498</td>
<td>80 (18)</td>
<td>901</td>
<td>153 (17)</td>
<td>320</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>402</td>
<td>290 (72)</td>
<td>627</td>
<td>194 (31)</td>
<td>150</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>693</td>
<td>333 (51)</td>
<td>1407</td>
<td>197 (14)</td>
<td>160</td>
</tr>
</tbody>
</table>

Table 9  Percentage quinolone resistance in main centres in South Africa

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>National Surveillance Academic Hospitals</th>
<th>Private Sector Surveillance</th>
<th>Johannesburg Hospital</th>
<th>Baragwanath Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Blood cultures)</td>
<td>(All Specimens)</td>
<td>(All specimens)</td>
<td>(All specimens)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>n = 1298</td>
<td>n=1399</td>
<td>n = 6834</td>
<td>n = 5372</td>
</tr>
<tr>
<td></td>
<td>2.9%</td>
<td>3.5%</td>
<td>3.9%</td>
<td>4.8%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>n = 289</td>
<td>n=332</td>
<td>n = 2079</td>
<td>n = 1996</td>
</tr>
<tr>
<td></td>
<td>2.4%</td>
<td>10.8%</td>
<td>15%</td>
<td>18%</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>n = 147</td>
<td>-</td>
<td>n = 1521</td>
<td>n = 451</td>
</tr>
<tr>
<td></td>
<td>35%</td>
<td>-</td>
<td>46%</td>
<td>52%</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>n = 2830</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>23%</td>
<td>-</td>
</tr>
</tbody>
</table>

(a) E. coli and Klebsiella only
(b) Dash (-) denotes that isolates were not routinely tested for quinolone susceptibility or not analysed
Table 10  Resistance in salmonellae and shigellae in South Africa\(^a\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Region</th>
<th>Total</th>
<th>Fluoroquinolones</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonellae (non-typhoid)</td>
<td>Excluding</td>
<td>606</td>
<td>0</td>
<td>Amp(^b) 35 Cot(^b) 23 Ceftr(^b) 2</td>
</tr>
<tr>
<td></td>
<td>KwaZulu Natal</td>
<td></td>
<td></td>
<td>Nalidixic acid 7.8 (Extended (\beta)-lactamase)</td>
</tr>
<tr>
<td>Salmonellae (non-typhoid)</td>
<td>KwaZulu Natal</td>
<td>89</td>
<td>0</td>
<td>Chlor(^b), Amp, Cot, Ceftr 0</td>
</tr>
<tr>
<td>S. typhi</td>
<td>All</td>
<td>268</td>
<td>0</td>
<td>Nalidixic acid 0.5</td>
</tr>
<tr>
<td>Shigellae</td>
<td>All</td>
<td>411</td>
<td>0</td>
<td>Nalidixic acid 0.5</td>
</tr>
<tr>
<td>S. dysenteriae type 1</td>
<td>KwaZulu Natal</td>
<td>354</td>
<td>0</td>
<td>Nalidixic acid 0.8</td>
</tr>
<tr>
<td>S. dysenteriae type 1</td>
<td>W Cape</td>
<td>38</td>
<td>0</td>
<td>Nalidixic acid 2.6</td>
</tr>
</tbody>
</table>

(a) Of 347 salmonellae tested in animals 5 (%4%) were resistant to fluoroquinolones
(Dr M M Henton, Onderstepoort Veterinary Institute)

(b) Amp = ampicillin, Cot = cotrimoxazole, Ceftr = ceftriaxone, Chlor = chloramphenicol
6 RESISTANCE TO QUINOLONES IN ZOONOTIC BACTERIA IN CHINA

Jin Shaohong

Although quinolones are relative new comers in the antimicrobial families they have been extensively used in human medicine and veterinary medicine. Because of their broad-spectrum of antimicrobial activity, good pharmacodynamics, safety and convenience, in China, from the first generation nalidixic acid, second generation pipemidic acid to the third generation such as norfloxacin, ciprofloxacin, ofloxacin, and sparfloxacin, more than 15 quinolones have been approved for clinical use by the drug regulatory agency since the 1980's. Norfloxacin, ciprofloxacin, ofloxacin, pefloxacin and enrofloxacin also have been approved by the ministry of agriculture for therapeutic use in food-producing animals.

Since antimicrobial resistance has become a major medical and public health problem, the main factor responsible for development and spread of bacterial resistance is injudicious use of antimicrobial agents, which has resulted in most gram-positive and gram-negative bacteria continuously developing resistance to the antimicrobials in regular use. In order to slow and control the emergence and spread of the antimicrobial resistance, the National Center for the Surveillance of Antimicrobial Resistance (NCSAR) was set up in the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) by the Ministry of Health, China in 1985. Since 1988 the NCSAR as the focal point of antimicrobial resistance monitoring (ARM) in China has been exchanging monitoring data with the Western Pacific Regional Office of WHO. In this presentation, the susceptibility of more than 17,000 clinical isolates collected from 11 hospitals in the Beijing area for the successive 4 years from 1994 to 1997 was tested by the Kirby-Bauer disk diffusion method following the NCCLS recommendations.

The results are shown in the table. The tested strains were Salmonella spp., Enterobacter spp., Shigella spp., Ps. aeruginosa, Enterococci, Staph. aureus, Staph. epidermidis and Streptococci, most of which are pathogens attacking both humans and animals. The tested drugs were ciprofloxacin, norfloxacin and ofloxacin.

According to the results in the table, the resistance of E. coli to quinolones has reached a rather high level. The resistant rates to ciprofloxacin were more than 50% in successive 4 years, compared to the 95% sensitivity rate reported by Thornsberry C in 1994, 100% sensitivity rate by Fass RJ in USA between 1986 and 1993, and 99% by Blondeau JM in Canada in 1996. Comparatively, the resistant rate of E. coli to quinolones in the Beijing area was much higher than that in other countries.
## Table: Rate of Resistance to Quinolones in China

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td>Ciprofloxacin</td>
<td>55% (1060)</td>
<td>54% (1128)</td>
<td>53% (875)</td>
<td>57% (1101)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>60% (10)</td>
<td>35% (307)</td>
<td>58% (263)</td>
<td>42% (514)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>58% (36)</td>
<td>50% (12)</td>
<td>44% (104)</td>
<td>54% (803)</td>
</tr>
<tr>
<td><strong>Salmonella spp.</strong></td>
<td>Ciprofloxacin</td>
<td>0% (16)</td>
<td>0% (3)</td>
<td>0% (1)</td>
<td>0% (2)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>100% (1)</td>
<td>0% (20)</td>
<td>0% (1)</td>
<td>0% (1)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>0% (2)</td>
<td>0% (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Enterococcus</strong></td>
<td>Ciprofloxacin</td>
<td>39% (565)</td>
<td>57% (7)</td>
<td>24% (225)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>100% (1)</td>
<td>32% (25)</td>
<td>49% (51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>50% (2)</td>
<td>20% (208)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Ciprofloxacin</td>
<td>18% (1311)</td>
<td>14% (1685)</td>
<td>13% (1212)</td>
<td>13% (1437)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>0% (2)</td>
<td>41% (75)</td>
<td>23% (111)</td>
<td>26% (323)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>23% (39)</td>
<td>27% (11)</td>
<td>30% (161)</td>
<td>24% (1122)</td>
</tr>
<tr>
<td><strong>Enterobacter spp.</strong></td>
<td>Ciprofloxacin</td>
<td>14% (474)</td>
<td>15% (60)</td>
<td>2% (57)</td>
<td>13% (217)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>25% (8)</td>
<td>28% (192)</td>
<td>12% (95)</td>
<td>0% (2)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>6% (16)</td>
<td>13% (8)</td>
<td>2% (42)</td>
<td>13% (215)</td>
</tr>
<tr>
<td><strong>Shigella dysenteriae</strong></td>
<td>Ciprofloxacin</td>
<td>0% (7)</td>
<td></td>
<td></td>
<td>0% (1)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>0% (6)</td>
<td>8% (24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>0% (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shigella flexneri</strong></td>
<td>Ciprofloxacin</td>
<td>1% (70)</td>
<td>7% (97)</td>
<td>6% (18)</td>
<td>5% (62)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>6% (153)</td>
<td></td>
<td></td>
<td>9% (136)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
<td></td>
<td></td>
<td>4% (27)</td>
</tr>
<tr>
<td><strong>Shigella sonnei</strong></td>
<td>Ciprofloxacin</td>
<td>0% (16)</td>
<td>0% (3)</td>
<td>3% (32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>0% (2)</td>
<td></td>
<td>0% (44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
<td></td>
<td>4% (75)</td>
<td></td>
</tr>
<tr>
<td><strong>Staph. aureus</strong></td>
<td>Ciprofloxacin</td>
<td>39% (603)</td>
<td>31% (573)</td>
<td>38% (519)</td>
<td>30% (719)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>0% (3)</td>
<td>0% (3)</td>
<td>43% (221)</td>
<td>25% (262)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>25% (4)</td>
<td>0% (1)</td>
<td>39% (59)</td>
<td>24% (486)</td>
</tr>
<tr>
<td><strong>Staph. epidermidis</strong></td>
<td>Ciprofloxacin</td>
<td>34% (615)</td>
<td>33% (666)</td>
<td>32% (609)</td>
<td>22% (660)</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>100% (1)</td>
<td>0% (2)</td>
<td>37% (126)</td>
<td>29% (282)</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>0% (2)</td>
<td></td>
<td>26% (95)</td>
<td>21% (474)</td>
</tr>
<tr>
<td><strong>Streptococci</strong></td>
<td>Ciprofloxacin</td>
<td>14% (126)</td>
<td>100% (1)</td>
<td>21% (34)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>0% (1)</td>
<td></td>
<td>17% (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>0% (1)</td>
<td></td>
<td>7% (29)</td>
<td></td>
</tr>
</tbody>
</table>
In our monitoring program, the data about some foodborne bacteria, such as *Salmonella* spp and *Shigella* spp, were not very integrated, however, these strains maintained a low level resistance to quinolones. *Shigella flexneri* resistance to quinolones was between 1% and 9% during the period from 1994 to 1997 in Beijing, which was similar to the reports of 2.1% from Hong Kong in the period of 1994-1995 (2) and 0.3-2.1% from the UK during 1991 to 1994 (4). Wiström J et al. reported that Shigellic resistance to quinolones was very rare in most regions in the world, which implied that the resistance rate in China was comparatively rather high, and we should be aware of this tendency.

Resistance of *Ps. aeruginosa* to quinolones was relatively at a stable level, similar to the reports from other countries, such as a 7.8% rate of resistance in the USA in 1990-1991 (5) and 79% susceptible in Canada in 1996 (1).

Enterococci are of nature high resistance characteristics. Under the great pressure of wide use of quinolones in human and/or food animals in China, the resistance to ciprofloxacin increased from 24% to 39% during this period. Similarly, Fass RJ reported that the susceptibility of *E. faecalis* to ciprofloxacin decreased from 95% in 1988 to 64% in 1993. *E. faecium* from 61% to 27%. This information indicated that the quinolones might be able to induce Enterococci to develop resistance.

In our surveillance data, both *Staph. aureus* and *Staph. epidermidis* were resistant to ciprofloxacin in the level of 30-40%, and were rising year by year. In contrary, a report from Canada (1), *Staph. aureus* was susceptible to ciprofloxacin in the rate of 96% in 1996.

From the data shown in the table, it could be said that resistance to quinolones in zoonotic bacteria in China was rather high, which could be related to the broad use and overuse of quinolones in humans and food animals. But so far, we have not had the direct experimental evidence to verify that the use of quinolones in food animals is definitely contributing to the resistance changes of isolates from humans.

So, it is our intention to:

1. Reinforce current ARM in China:

   1.1 Establishing a special program for zoonotic bacteria, such as *Salmonella* spp, *Campylobacter* spp, etc.

   1.2 Enlarging the surveillance network in China.

   1.3 Cooperating with different departments: drug administration, medical service, pharmaceutical companies, and agriculture department.

2. Develop a research project on finding direct evidence of whether the same resistance genes exist in strains from humans and food animals.

3. Formulate guidelines for rational use of antimicrobial agents in humans and food animals.
References


7 REVIEW OF QUINOLONE RESISTANCE IN HUMAN PATHOGENS

Yunsop Chong and Kyungwon Lee

New quinolones are very useful antimicrobial agents for the treatment of infections including those due to the pathogens resistant to various antimicrobial agents. Fluoroquinolones have been used to treat the infections of urinary, respiratory and gastrointestinal tracts, of skin, soft tissue and bone, as well as sexually transmitted diseases.

As was with other antimicrobial agents, it was hoped that the high activity of the new broad-spectrum quinolones could last long. It was considered that emergence of resistance to fluoroquinolone is substantially lower compared to nalidixic acid (1). Quinolones are capable of inhibiting the conjugative transfer of plasmids. In fact fluoroquinolones may cure some R plasmids.

It was just ten years before when Davies (2) stated that fluoroquinolones were extremely promising compounds, but prudent use would be an important measure of guaranteeing their maximal benefit in future clinical practice. Although quinolone resistance rate may vary greatly depending on the usage of the agents, on the species and clinical settings, rapid increase of resistant bacteria have been noted in many countries. In this review the serious nature of clinically relevant quinolone resistance will be presented briefly.

Enterobacteriaceae

Escherichia coli and other species of Enterobacteriaceae are the most frequent causes of infections. The frequency of selection of spontaneous single step mutations that confer an 8-fold or greater increase in drug resistance in E. coli was reported to be several orders of magnitude lower with the newer fluoroquinolones than with nalidixic acid. However, during the treatment of urinary tract infections, resistance had occurred in 2.9% of patients receiving ciprofloxacin. Development of resistance was more frequent, i.e., 27%, in patients with complicated urinary tract infections (7).

In the United States (3) and Canada (4), fluoroquinolone resistance rates of Enterobacteriaceae remained low in the early 1990 (Table 1). In Spain, E. coli resistant to pipemidic acid was less than 6% prior to 1989, but increased to 18% in 1992. The resistance rate to ciprofloxacin increased from 4% in 1991 to 7.1% in 1992. The increase of resistance was coincided with higher consumption of fluoroquinolones (5).
Table 1  Fluoroquinolone resistance rates of gram-negative bacilli isolated in different countries

<table>
<thead>
<tr>
<th>Organism</th>
<th>United States</th>
<th>Canada</th>
<th>Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>28,805 2</td>
<td>946 1</td>
<td>818 5</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>9,773 3</td>
<td>355 4</td>
<td>1,031 1</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>2,158 5</td>
<td>84 12</td>
<td>193 3</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>5,170 1</td>
<td>181 3</td>
<td>783 18</td>
</tr>
<tr>
<td>Serratia</td>
<td>2,846 4</td>
<td>61 8</td>
<td>347 11</td>
</tr>
<tr>
<td>Morganella</td>
<td>1,610 &lt;1</td>
<td>39 3</td>
<td>170 8</td>
</tr>
<tr>
<td>Proteus</td>
<td>7,001 &lt;1</td>
<td>128 2</td>
<td>322 0</td>
</tr>
<tr>
<td>Providencia</td>
<td>677 2</td>
<td>14 38</td>
<td>17 3</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>2,216 17</td>
<td>49 22</td>
<td>747 25</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>16,206 8</td>
<td>390 21</td>
<td>2,331 24</td>
</tr>
</tbody>
</table>


*b Blondeau et al., 1996. Includes intermediate susceptibility.

In a tertiary care hospital in Korea, fluoroquinolones became increasingly used since early 1990s. In 1991, the rate of resistance of E. coli was 5%, but in 1994 it rose to 26% and then to 39% in 1997. Klebsiella pneumoniae is a frequently isolated organism, too. As this species are often resistant to extended-spectrum beta-lactams, fluoroquinolones are good alternatives. Although the fluoroquinolone resistance rate was lower than that of E. coli, the rate became over 10% since 1994. High resistance rates were also noted in Citrobacter freundii and Serratia marcescens.

Glucose-nonfermenting gram-negative bacilli

Pseudomonas aeruginosa and Acinetobacter baumannii are frequent cause of nosocomial infections. The resistance rate of P. aeruginosa was 24% already in 1991 in Korea. It rose to 41% in 1994 and then 50% in 1997. The resistance rate of A. baumannii was 64% in 1997. Emergence of resistance occurs more often with P. aeruginosa as is with S. aureus (1). It is because quinolones are prone to select multidrug resistance (MDR) phenotype in P. aeruginosa. The mutations result in over expression of multidrug efflux system (6).

As is with other antimicrobial resistance, the fluoroquinolone resistance rate was much higher for the strains isolated from intensive care unit patients (Table 2). Only 36% of the E. coli isolated from ICU patients were susceptible to fluoroquinolones, while the rate was 63% for the strains isolated from other patients. The rates in both patients groups were similar for P. aeruginosa, probably because the patients acquire the infection almost always nosocomially.

Antimicrobial resistant organisms are more prevalent in large tertiary care hospitals. A nationwide survey in 1994 to 1995 in Korea showed that fluoroquinolone resistant bacteria were not rare in smaller hospitals (Table 3). This suggests that quinolone
Table 2  Comparison of fluoroquinolone susceptibility by patient group in 1996

<table>
<thead>
<tr>
<th>Species</th>
<th>Patient group</th>
<th>No. of isolates</th>
<th>% of isolates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
</tr>
<tr>
<td>E. coli</td>
<td>ICU</td>
<td>259</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Non-ICU</td>
<td>2,254</td>
<td>63</td>
<td>1</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>ICU</td>
<td>565</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Non-ICU</td>
<td>1,598</td>
<td>45</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 3  Comparison of fluoroquinolone resistance rates in different size of hospitals

<table>
<thead>
<tr>
<th>Species</th>
<th>No. tested</th>
<th>Resistance rate (%) in hospitals: a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S-L</td>
</tr>
<tr>
<td>E. coli</td>
<td>5,916</td>
<td>23</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>2,670</td>
<td>13</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>1,145</td>
<td>23</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>984</td>
<td>39</td>
</tr>
</tbody>
</table>

a Location: S, Seoul; NS, non-Seoul. Bed capacity: L, >1000; M, 500-1000; S, <500.

resistance is indeed a serious problem in some countries. Another problem is the biological cross-resistance between all fluoroquinolones. All gram-negative bacilli resistant to ciprofloxacin were also resistant to trovafloxacin (7).

**Enteric pathogens**

Diarrheal diseases caused more than 3 million deaths in 1995 worldwide (8). Fluoroquinolones are active against enteric pathogens such as *Salmonella*, *Shigella*, *Campylobacter* and *Vibrio* sp. Typhoid fever, a disease with high morbidity and mortality, occurs about 16 million cases a year. Recent increase of multiresistant *S. typhi* poses serious problem, as there are no effective alternative drugs for the treatment, except fluoroquinolones. It was in 1990 that Asperilla et al. (9) concluded in a review that fluoroquinolones were clinically effective against infections due to multiresistant *S. typhi* and were effective in eradicating the organism from biliary and fecal reservoirs. Also, contrary to other drugs, fluoroquinolones could shorten the clinical course of nontyphoidal *Salmonella* enteritis and terminate excretion of these organisms in the stool.

However, fluoroquinolone-resistant *S. typhi* started to appear (10). In Viet Nam, the nalidixic acid-resistant *S. typhi* was detected in 1993. It was reported that among the 150 strains of *S. typhi* isolated in 1993-1994, 78% were multiresistant and among the resistant ones 15% were resistant to nalidixic acid (Table 4). Some of the infections due to nalidixic acid-resistant strains responded unsatisfactorily to ofloxacin treatment (11). The problem with the susceptibility test was that nalidixic acid-resistant strains were inhibited by <1 µg/ml
of ofloxacin, and 5-µg disk may not differentiate nalidixic acid-susceptible isolates from resistant ones. Increase of quinolone-resistant *S. typhi* may become a serious problem in the future. Thralfall et al. (1997) expressed concern about the substantial increase of ciprofloxacin-resistant *S. typhimurium, S. virchow* and *S. hadar* since 1994 in the United Kingdom (12).

*Shigella* species isolated in the United Kingdom, in 1995 to 1996, were often resistant to ampicillin and trimethoprim, but the resistance rate to ciprofloxacin was 0.2% to 0.5% depending on the species (13). The resistance seems to be rare at the moment.

*Campylobacter* is the most common or the second most common enteric pathogen in many countries. The organism was very susceptible to various antimicrobial agents. The resistance rates of *Campylobacter* vary greatly depending on the region of isolation. Among the 722 *C. jejuni* strains isolated in America in 1996, only 5% were resistant to both nalidixic acid and ciprofloxacin. Most of the patients had history of travel to foreign countries (14). However, in Spain, Sanchez et al. (15) reported a striking increase of resistance in 1991 (Table 5). Recently, Perez-Trallero et al. (16) reported the fluoroquinolone susceptibility of 4261 isolates of *C. jejuni/coli* from man in Spain in 1981-1996. Until 1990, the nalidixic acid resistance rate was about 1%. However, from 1990 to 1991 the resistance rate rose from 6.8% to 29%. Most nalidixic acid-resistant strains were also resistant to ciprofloxacin. In 1996, the resistance rate to ciprofloxacin was 81.6%. The investigators considered that the consumption of quinolones only in humans could hardly explain this high prevalence of resistant strains.

### Table 4

<table>
<thead>
<tr>
<th>Year of isolation</th>
<th>No. tested</th>
<th>No (%) of isolates resistant to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Multidrug</td>
</tr>
<tr>
<td>1992-1993</td>
<td>41</td>
<td>26 (63)</td>
</tr>
<tr>
<td>1993-1994</td>
<td>150</td>
<td>117 (78)</td>
</tr>
<tr>
<td>1995</td>
<td>720</td>
<td>662 (92)</td>
</tr>
</tbody>
</table>

*Wain et al., 1997.

### Table 5

<table>
<thead>
<tr>
<th>Year of isolation (No. tested)</th>
<th>Ciprofloxacin (µg/ml)</th>
<th>Ofloxacin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>1988 (38)</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>1989 (47)</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>1990 (46)</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>1991 (63)</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>1992 (81)</td>
<td>1</td>
<td>32</td>
</tr>
</tbody>
</table>

*Sanchez et al., 1994.
**Staphylococcus aureus**

*S. aureus* remains one of the most important pathogens causing community-acquired as well as nosocomially-acquired infections. MIC\(_{\text{ew}}\) of ciprofloxacin for MSSA was less than 1 μg/ml (17). Among the isolates in the hospitals in Korea and Japan MRSAs were more prevalent than MSSAs and the former were often multiresistant to various antimicrobial agents except to glycopeptides (18). The resistance rate to fluoroquinolone was very similar to that to methicillin (Table 6).

*S. aureus* is resistant to fluoroquinolone by three mechanisms: mutations in the genes encoding the DNA gyrase (*gyrA*) and topoisomerase IV A subunit (*girA*), the gyrase B-subunit gene (*gyrB*), and over expression of *norA* (*NorA*: multidrug efflux protein). The mechanism can exist singly or in combination (19).

Some of the newer fluoroquinolones are more active against gram-positive aerobes or anaerobes. For example, some of the *S. aureus* isolates and enterococci with high level resistance to ciprofloxacin were borderline susceptible to trovafloxacin (7).

**Streptococcus pneumoniae**

Pneumococcal pneumonia is also prevalent disease (8). Pneumococci are also a leading cause of bacterial meningitis. Increase of penicillin-resistant pneumococci has been observed in many countries. The MIC\(_{\text{ew}}\) of ciprofloxacin for clinical isolates of *S. pneumoniae* is relatively high, i.e., 1 to 3 μg/ml. Mutant pneumococci resistant to ciprofloxacin were not resistant to grepafloxacin (20). However, in general there were cross-resistances between fluoroquinolones. The activity of multidrug efflux transporter is likely to contribute to the intrinsically low sensitivity of this organism to fluoroquinolones (21). Ciprofloxacin has not been proven to be consistently reliable for treating pneumococcal disease. The newer fluoroquinolones such as grepafloxacin and trovafloxacin are more active against pneumococci in vitro (Table 7). Whether these differences in in vitro potency offer any

### Table 6  Multiresistance of *Staphylococcus aureus* isolated in 1994 in Korea *

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>MSSA No.</th>
<th>MSSA %</th>
<th>MRSA No.</th>
<th>MRSA %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pen Ery Tet Pef Cln Fus Sxt</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.4</td>
</tr>
<tr>
<td>Pen Ery Tet Pef Cln Sxt</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>5.7</td>
</tr>
<tr>
<td>Pen Ery Tet Pef Cln Fus</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>2.6</td>
</tr>
<tr>
<td>Pen Ery Tet Pef Cln</td>
<td>3</td>
<td>0.8</td>
<td>443</td>
<td>60.0</td>
</tr>
<tr>
<td>Pen Ery Tet Pef</td>
<td>9</td>
<td>2.4</td>
<td>61</td>
<td>8.3</td>
</tr>
<tr>
<td>Other 4 to 6</td>
<td>26</td>
<td>6.9</td>
<td>82</td>
<td>11.1</td>
</tr>
<tr>
<td>Less than 4</td>
<td>338</td>
<td>89.9</td>
<td>88</td>
<td>11.9</td>
</tr>
</tbody>
</table>

Total 376 100 738 100

* Abbreviations: Pen, penicillin G; Ery, erythromycin; Tet, tetracycline; Pef, pefloxacin; Cln, clindamycin; Fus, fusidic acid; Sxt, cotrimoxazole.

---

Use of Quinolones in Food Animals and Potential Impact on Human Health 85
Table 7  In vitro activities of fluoroquinolones against 190 isolates of
*Streptococcus pneumoniae*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5-4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>0.5-2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>2-16</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Tosufloxacin</td>
<td>0.06-0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Visali et al., 1996.*

important clinical advantage remains unknown (22).

**Neisseria gonorrhoeae**

It is estimated that 62 million cases of gonorrhoea occurred in 1995 (8). Fluoroquinolones were very active against gonococci, and initially the development of resistance in *Neisseria* was rare. It has been a recommended drug for the treatment of gonorrhea, particularly when the penicillinase-producing strains are prevalent. However, rapid increase of resistant isolates was reported lately in Southeast Asia (23). In Korea around 70% of the isolates from surveillance cultures were penicillinase-producing strains (Table 8). Fluoroquinolone-susceptible isolates decreased from 91% in 1992 to 33% in 1997. Although most of the non-susceptible isolates were intermediate rather than resistant, fully resistant strains may increase in the near future.

**Summary**

The rapid increase of fluoroquinolone-resistant bacteria have been noted in many countries. In some countries, the resistance rates of some species such as *E. coli*, *C. freundii*, *P. aeruginosa*, *A. baumannii* and *S. aureus* are so high that empirical selection of fluoroquinolone is difficult. Although the magnitude may vary greatly depending on various situations, the increasing resistance is defying our hope to extend the effect of the drug. Increased use of fluoroquinolone should be the direct cause of the emergence of resistant organisms and the dissemination of the resistant clones further increases the resistant bacteria. We should come up with certain measures to retard further emergence and spread of the fluoroquinolone-resistant pathogens.

Table 8  Antimicrobial susceptibility by disk diffusion test of *Neisseria
gonorrhoeae* isolated in Korea

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-lactamase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>70</td>
<td>71</td>
<td>70</td>
<td>76</td>
<td>70</td>
<td>79</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>susceptible</td>
<td>91</td>
<td>76</td>
<td>68</td>
<td>58</td>
<td>46</td>
<td>33</td>
</tr>
</tbody>
</table>

*No. in parentheses indicates no. of isolates tested. All isolates were susceptible to ceftriaxone and spectinomycin.*

86  Use of Quinolones in Food Animals and Potential Impact on Human Health
References


8 Initial Attempts to Assess the Environmental Fate of the Veterinary Fluoroquinolone Enrofloxacin by In Vitro Degradation Studies Employing Basidiomycetous Fungi Isolated from Wood, Agricultural Soils, and Cattle Dung

H-G Wetzstein and N Schmeer

The first fluoroquinolone (FQ) developed for veterinary application, enrofloxacin (17), is now registered in many countries and used to treat serious bacterial infections in pets and livestock animals, e.g., bronchopneumonia or diarrhoea in calves and pigs. Other FQs currently applied in veterinary medicine comprise danofloxacin, difloxacin, marbofloxacin, orbifloxacin, and sarafloxacin. Little information is available regarding the metabolites formed from these drugs in the course of their passage through target animals. However, FQs used in human medicine have been studied in more detail in various species of laboratory animals, in which FQs were found to be metabolized - often only to a limited extent - by (i) (reversible) glucuronidation and sulfation, (ii) oxidation of the amine moiety, and (iii) dealkylation of an appropriate amine moiety (4). In the latter reaction, e.g., ciprofloxacin is formed from enrofloxacin in cattle (17, 24); the same reaction has been observed with pefloxacin (yielding norfloxacin) and with ofloxacin (4). Degradation of the heterocyclic core of a FQ in animals has not been reported. Hence, a fraction of the dose of an applied FQ will enter the environment as unchanged parent drug in urine and feces. Especially, the application of FQs in livestock animals may raise concerns due to the higher absolute amount of drug needed for the treatment in one location. Furthermore, after a period of storage manure of, e.g., cattle and poultry (which is usually mixed with straw or wood shavings, respectively), is often disposed of by spreading onto agricultural fields and pastures as fertilizer. In this way, drug residues might be distributed more widely in the environment.

FQs were shown to be bound by human feces (6, 14) and soil (9, 12, 18), whereby their bioavailability was strongly reduced. Information regarding the binding to animal dung
is not available to our knowledge. In the presence of 30% human feces, the minimal bactericidal concentration of ciprofloxacin against *Acinetobacter sp.*, *Escherichia coli*, and *Pseudomonas aeruginosa*, was increased by 300 to 800-fold (14). In a model simulating the human gut, *E. coli* isolates were found to be about 5-fold less sensitive to sarafloxacin than in broth (3). Moreover, concentrations of 1000 times the MIC of sarafloxacin were reported to have had no effect on the viability of bacteria from a sediment (18). Unspecific binding to soil restricts the mobility of FQs in the environment, which may be a quite favorable characteristic of a substance (8), although such binding may contribute to the apparent recalcitrance of FQs. It is tempting to speculate that under such conditions only a low (possibly no) pressure to select for resistance may be exerted. However, definitive information is lacking, so far. Because of a fluoro-aromatic structural element, which on the one hand strongly enhances their antibacterial potential but on the other hand has not been found to occur in nature, FQs are regarded as xenobiotics.

**Degradation of FQs in solid matrices**

To evaluate whether enrofloxacin was biodegradable in *vitro* studies were initiated. It could be demonstrated that enrofloxacin was decomposed by several fungal species belonging to the Basidiomycetes (10). Four wood-degrading species, representing both the so-called white rot and brown rot fungi, were found to mineralize enrofloxacin: Within 8 weeks, up to 50% $^{14}$CO$_2$ was released from [carbonyl-$^{14}$C]enrofloxacin, if the drug had been adsorbed to autoclaved wheat straw. If the drug had been pre-bound to gamma-sterilized agricultural soil, the amount of $^{14}$CO$_2$ formed from enrofloxacin by *Gloeophyllum striatum* was still 6% within 15 weeks (10). This proved that enrofloxacin was degraded, even in the soil-bound state. The maximum rate of $^{14}$CO$_2$ formation in gamma-sterilized soil reached 0.9% per week. This is similar to degradation rates observed with a community of soil microorganisms degrading a synthetic humic acid complex containing $^{14}$C-labeled 3,4-dichloroaniline (15). Gamma-irradiated soil was used to prevent likely interactions between *G. striatum* and the native soil microorganisms, which is an additional parameter determining the apparent degradation activity in an *in vitro* test system. Such a population dynamics effect was then demonstrated by adding native soil (containing bound enrofloxacin) to an active straw culture of *G. striatum*: $^{14}$CO$_2$ formation was drastically reduced within one week, indicating that the soil population had gained control under the specific conditions in this model ecosystem (10).

Natural degradation rates of humus are reported to be in the order of 2 to 5% per year (11). If FQs were bound to the humus fraction, degradation rates in this order of magnitude are to be expected. Under *in vitro* conditions using one authentic agricultural soil supplemented with 10 ppm of [carbonyl-$^{14}$C]enrofloxacin, the apparent rate of $^{14}$CO$_2$ formation was 0.1% per 8 weeks (10). Similar degradation rates were observed with three different soils containing sarafloxacin: after 80 days, up to 0.6% $^{14}$CO$_2$ was produced from [2-$^{14}$C]sarafloxacin (9,18).

If the rate of $^{14}$CO$_2$ formation from [carbonyl-$^{14}$C]enrofloxacin bound to soil (0.1%
per 8 weeks) is compared with the maximum rate observed on straw (17% per week), the potential extent of variation within in vitro degradation rates (here 1000-fold) is obvious (10). Hence, rates of \(^{14}\text{CO}_2\) formation have to be interpreted with care. They might not correlate well with turn-over and residual biological activity of drug residues in the matrix. Although, no rates of \(^{14}\text{C}\text{O}_2\) production were reported for dano- floxacin (2), estimated times of 50% transformation of its amine moiety in three soils were mentioned in that paper: these were in the order of 87 to 143 days (2), which matches the time we observed for such degrada- tion of enrofloxacin on straw by a native population of soil microbes (22). Only one report is available on the fate of a FQ, sarafloxacin, in a marine sediment. Its apparent depuration was attributed to leaching and redistribution rather than to degradation (7).

Degradation in a defined mineral medium

Because the brown rot fungus \(G. \text{striatum}\) had shown highest activity on straw (10), it was subsequently studied in more detail employing liquid cultures. In a defined mineral medium, mycelia transformed 10 ppm of enrofloxacin quantitatively into metabolites within about one week (20). At that point in time, as little as 5% \(^{14}\text{CO}_2\) had been formed from [carbonyl-\(^{14}\text{C}\)]enrofloxacin and even less, 1% \(^{14}\text{CO}_2\), had been formed from [piperazine-2,3-\(^{14}\text{C}\)]enrofloxacin in parallel cultures. Unspecific binding of the label to \(G. \text{striatum}\) could be eliminated as one potential source of interference: It was in the order of 5%, indicating a full bioa- vailability of the drug and its metabolites. Clearly, the proportion of \(^{14}\text{CO}_2\) formed did not reflect the extent of transformation (in this case of degradation) the drug molecule had already undergone. The amount of \(^{14}\text{CO}_2\) rather indicated the minimum level of destruction. Quantitative transformation of the drug was correlated with 5% \(^{14}\text{CO}_2\) produced from the carbonyl label. The very small amount of \(^{14}\text{CO}_2\) liberated from the piperazine moiety was an inferior indicator of turnover under these conditions. De- grada- tion of the amine moiety of danofloxacin by a wide taxonomic range of soil microor- ganisms has been studied in a complex li- quid medium ([2], see below). In addition, the formation of 31% \(^{14}\text{CO}_2\) (or other volatile components) from [2-\(^{14}\text{C}\)]danofloxacin has been observed after 24 hours with one strain of \(Curvularia\ lunata\), an Ascomycete (2).

Metabolites formed by fungi and bacteria

All metabolites detected in the supernatant of liquid cultures of \(G. \text{striatum}\) appeared only transiently. The pattern of major metabolites indicated that maximum concentrations in the order of 10% were attained already after 2 days of incubation. These metabolites were purified by preparative HPLC. Their molecular structures were elucidated combining HPLC-mass spectrometry and, in most cases, \(^1\text{H}\) NMR spectroscopy. All of the eleven identified metabolites of enrofloxacin (20) and, only recently, of ciprofloxacin (19), had essentially lost their antibacterial activity and appeared to be quite unstable. They were grouped into four categories: (i) mono-hydroxylated congeners, (ii) dihydroxylated congeners, (iii) metabolites indicating the cleavage of the heterocyclic core, and (iv) metabolites demonstrating both the elimination
and the oxidative degradation of the amine substituent. The most important monohydroxylated metabolites are F-1, an oxidatively decarboxylated congener, and F-2, in which fluorine was replaced by a hydroxyl group (20). F-1 can be expected to have no residual antibacterial activity because the carboxyl group is essential for antibacterial activity of FQs (5). The residual activity of metabolites containing an oxidized amine moiety appears to be in the order of less than 3% (4, 20, 22). This pattern of metabolites, recently confirmed with ciprofloxacin (19), was postulated to be generated through the action of hydroxyl radicals, possibly in a Fenton-type reaction (20). Other mechanisms, involving, e.g., aryloxy radicals and Mn⁶⁺, have been described to occur in white rot Basidiomycetes being employed for the degradation of lignin and a wide range of pollutants (1). Based on our results regarding the degradation of enrofloxacin (10, 20), more than one mechanism can be expected to be operative in nature for the degradation of FQs. Diffusible radicals provide the principle means for the degradation of compounds adsorbed to dung and soil matrices. Thus, in contrast to earlier views (15), at least a fraction of an adsorbed substance is accessible to degradation by such fungi.

Deethylation and, more importantly, the formation of the 7-amino congener of danofoxacin by bacteria (Pseudomonas fluorescens, Mycobacterium spp.), the yeast Candida lipolytica, and three species of the mould Penicillium (Deuteromycetes), indicates the presence of a broad potential in nature for the degradation of the amine moiety, leading to inactivation of the antibacterial potential of FQs (2).

Approaching in situ conditions

Basidiomycetes with physiological properties resembling those of wood-rotting species are also found as part of the coprophilous microflora in aged cattle dung (23). Consequently, we assessed the potential of such species for the degradation of enrofloxacin (22) and ciprofloxacin (21). Strains of Cyathus stercoreus and Panaeolus sphinctrinus as well as one strain of Psilocybe cubensis were tested in liquid cultures and on autoclaved aged cattle dung. Degradation kinetics were recorded by following ¹⁴CO₂ production from [carbonyl-¹⁴C]- and [piperazine-2,3-¹⁴C]ciprofloxacin. After two weeks, up to 12% ¹⁴CO₂ was produced, indicating the presence of a degradation potential in these species. It was concluded that regular members of the fungal community found in cattle dung are able to degrade enrofloxacin and ciprofloxacin in situ (21).

Relatively little is known about lignocellulose-degrading Basidiomycetes from soil (16). However, several species of three genera of such fungi (Agrocybe, Cyathus, Hyphnum) were recently identified to degrade ciprofloxacin ¹⁴C-labeled either at the carbonyl-C or in the piperazine moiety (21). Lignin degradation is also found in other species of Ascomycetes and Deuteromycetes derived from soil. Although, these groups will not be discussed here (the reader is directed to the literature compiled in [13]), they suggest a wider potential to exist in nature for the degradation of FQs. Furthermore, preliminary results from studies in our laboratory have demonstrated, that a high degradation potential for enrofloxacin prevails in the indigenous population of microorganisms in rotting wheat straw,
which was recovered from agricultural soil (22). Here, the initial rate of \(^{14}\text{CO}_2\) formation from [piperazine-2,3 \(^{14}\text{C}\)]enrofloxacin was found to be approximately 5% per month, reaching up to 25% after 7 months. In parallel cultures, an initial rate of only about 0.05 % \(^{14}\text{CO}_2\) from [carbonyl-\(^{14}\text{C}\)]enrofloxacin was observed per month. Comparing these initial rates, a 100-fold higher degradation activity was indicated by the piperazine label - signaling inactivation of the drug (22). Hence, both positions may differ considerably in their diagnostic value, depending on the experimental conditions. While \(^{14}\text{CO}_2\) formation from [piperazine-2,3 \(^{14}\text{C}\)]enrofloxacin probably indicates inactivation of the antibacterial activity, \(^{14}\text{CO}_2\) from the carbonyl position may indicate the (much) slower mineralization of the heterocyclic core of the drug.

Any risk assessment with a FQ would have to take into consideration (i) its most likely in situ concentration, (ii) the effect of unspecific binding on its bioavailability and on its potential residual biological activities, and (iii) an approximation of the kinetics of the loss of its antibacterial potential. Although significant progress has been made, due to limited data available, none of these points is fully understood at present. But on the other hand, there is no indication of a significant risk of FQs to the environment either.

**Conclusions**

We conclude that enrofloxacin, ciprofloxacin and, likewise, other FQs are biodegradable. Most likely, they are decomposed in situ. However, direct experimental evidence is difficult to obtain. Degradation is expected to be delayed by unspecific binding to organic matter like dung or by adsorption to soil. Many unknown parameters, not least, the heterogeneous nature of authentic matrices, will influence degradation rates in any particular experimental system. Presently, it is unclear under which conditions (if possible at all) a typical half-life of a FQ could be determined. Inactivation of the antibacterial activity of enrofloxacin was best demonstrated by following the release of \(^{14}\text{CO}_2\) from amine-labeled drug. Although little information is available concerning the residual activity of metabolites of FQs, such activities can be expected to be far below the potency level of parent drugs. Most of the identified metabolites produced by *G. striatum* (20) are thought to, essentially, have lost antibacterial activity. Metabolites containing an oxidized amine moiety have a residual activity in the order of less than 3% (20, 22). Therefore, they are valid indicators of the loss of antibacterial potential. In contrast, the kinetics of \(^{14}\text{CO}_2\) formation from positions within the heterocyclic core of a FQ might be more typical of the turnover rate of humus. Fluorine, the xenobiotic structural element was readily eliminated from enrofloxacin and ciprofloxacin by *G. striatum*. Various species inhabiting agricultural soil or cattle dung have been shown to possess a degradation potential for enrofloxacin and ciprofloxacin under our in vitro experimental conditions. However, more information is required regarding (i) degradation kinetics in natural matrices, and (ii) residual antibacterial activity of metabolites, to be able to fully describe the environmental fate and to assess the potential risks of FQs. Improved methods are needed to facilitate an analysis of the biodegradability of drug substances like FQs in an early phase of development.
References


9 OVERVIEW OF QUINOLONE USAGE FOR FOOD-PRODUCING ANIMALS

J van Diest and A de Jong

This paper presents a brief history of quinolones used in animals and elucidates several aspects of the current uses of fluoroquinolones, particularly in regards to the quantities produced and the geographic distribution. Differences in the conditions of use between developed and developing countries are pointed out. Also, usage in animals is compared with the use of fluoroquinolones in human medicine. Finally, comments are made on the future use of fluoroquinolones in animal health.

Nalidixic acid is the predecessor of all modern quinolones used in both human and animal medicine. The drug was discovered in the early sixties, although the nucleus of the molecule was known earlier in anti-malaria research programmes. Nalidixic acid, however, demonstrated no anti-malaria effect but did display interesting antibacterial activity. This drug was the first quinolone used clinically and was broadly administered to people and animals. Nowadays, there is very limited use of this drug because of its narrow spectrum (Gram-negative only), poor antimicrobial potency, inferior pharmacokinetic features, marked side effects and relatively high propensity to induce resistance.

Subsequent quinolones, all congeners of nalidixic acid and discovered in the late sixties and seventies, clearly showed both improved antibacterial and pharmacokinetic properties as well as reduced side effects. Some of these are still used in human medicine (e.g., pipemidic acid) and some others are being used in veterinary medicine (flumequine, oxolinic acid) in a limited number of countries.

A significant breakthrough was achieved by the discovery of the fluoroquinolones characterised by both a fluorine atom at the 6 position and an amine group (usually a piperazine moiety) at the 7 position. Currently several fluoroquinolones are available for therapy of animals in one or more countries. However, the usage of these fluoroquinolones differs greatly as regards animal species, indications, label conditions and geographic spread, which is summarised in Table 1 and Table 2. These tables do not include fluoroquinolones developed for human medicine which are additionally licensed for animals in very few countries, e.g., pefloxacin or ciprofloxacin (e.g., in China, Korea and Mexico).

While enrofloxacin has been registered for a wide range of domestic animals, others are approved for a few animal species; particularly fluoroquinolones in poultry medicine (Table 1). Recently new approvals were granted for companion animals (orbifloxacin, marbofloxacin, difloxacin) as well as for poultry (sarafloxacin, difloxacin), livestock (marbofloxacin) and salmon (sarafloxacin).
A number of fluoroquinolones are approved for use in livestock in Europe, Asia and Latin America, whereas one fluoroquinolone is approved in the USA and none in Australia (Table 2). In contrast, in all geographic areas, except Australia, fluoroquinolones are commercially available for poultry. With respect to dogs and cats enrofloxacin is used in all countries; in industrialised countries others are approved as well. Table 2 does not contain fluoroquinolones for aquaculture: for this indication mainly oxolinic acid is applied, e.g., in South Korea, Japan and other Asian countries.

Several formulations of the approved fluoroquinolones, both oral and parenteral, have been developed, depending on animal species. Major indications are respiratory and enteric infections in livestock and poultry, while dermal and urinary tract infections are frequently indicated for pets. Major bacteria to be treated are, for instance, Pasteurella spp., Haemophilus spp., Actinoba-
cillus pleuropneumoniae, Mycoplasma spp. or E. coli, along with Staphylococcus intermedius in dogs and cats. Dosages vary according to animal species, indication (i.e. causative bacteria) and quinolone. Generally, the dosage ranges from 1 and 5 mg/kg b.w. for livestock, amounts 5 or 10 mg/kg b.w. for poultry and is 2 to 5 mg/kg b.w. for dogs and cats. Duration of treatment is as a rule 3 to 5 days but can be longer especially with chronic dermal infections in dogs; route of administration is oral (mainly water medication and tablets), subcutaneously, intramuscularly or intravenously.

The usage of fluoroquinolones differs greatly among countries and geographical locations, which is not apparent from the above tables. For instance, whereas the marketing of a given fluoroquinolone is limited to one country and to one species, other fluoroquinolones are commercialised in various countries and for several indications. Published data on antibiotic consumption are

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Trade name</th>
<th>Cattle</th>
<th>Pigs</th>
<th>Chickens</th>
<th>Turkeys</th>
<th>Dogs</th>
<th>Cats</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrofloxacin</td>
<td>Baytril</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>Advocin / Advocid</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>Quinabic</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Oxaldin</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Vebufloxacin</td>
<td>Pipilocin</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Sarafloxacin</td>
<td>Floxasol / Saraflox / Sarafin</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Orbifloxacin</td>
<td>Victas / Orbax</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>Marbocyl</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
<tr>
<td>Difloxacin</td>
<td>Vetequinon / Dicural</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
<td>☒</td>
</tr>
</tbody>
</table>
very rare. Any attempt to estimate the total worldwide usage in value and weight is seriously hampered by the lack of reliable data from some parts of the world. It sounds so easy to obtain such data but in reality there are many hurdles to overcome, e.g., commercial confidentiality, parallel imports and generic manufacture by numerous and sometimes elusive companies. In addition, there is counterfeit and illegal manufacture as well as illegal use of human quinolones for animals.

Accurate figures or at least relatively reliable estimates about the usage of quinolones in animals (exclusive fish) were secured for 1997 for good 30 industrialised and developed countries, principally West and South Europe, North and Latin America, South Africa and major Asian countries (Japan, Malaysia, Philippines, South Korea, Taiwan). The total sales in value of the countries covered by this survey amounted to 175 million dollars and sales estimates increased to 191 million dollars if the second-generation quinolones flumequine and oxolinic acid are included. Large differences in proportions among the different quinolones were apparent. This analysis also points out the significance of generic quinolones, mainly enrofloxacin, in a number of Latin American and Asian countries as well as Spain. If the sales of these generics are included, total turnover is estimated at 239 million dollars. The majority is for use in poultry, about 20-25% of the sales is attributed to formulations for companion animals.

Total sales of all classes of antibacterial drugs for disease therapy in animals in these countries are estimated to approach 2 billion US dollars; hence, the proportion of quinolone sales is about 12%. Sales in weight are more meaningful as to emergence and spread of resistance than sales in value, but even more difficult to gather. The usage of fluoroquinolones for food-producing animals in the year 1997 is estimated to

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Fluoroquinolone approvals for domestic animals by geographic areas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Livestock</td>
</tr>
<tr>
<td>Europe</td>
<td>enrofloxacin, marbofloxacin, danofloxacin</td>
</tr>
<tr>
<td>USA</td>
<td>enrofloxacin</td>
</tr>
<tr>
<td>Japan</td>
<td>enrofloxacin, danofloxacin, orbifloxacin, difl oxacin</td>
</tr>
<tr>
<td>Asia</td>
<td>enrofloxacin, danofloxacin</td>
</tr>
<tr>
<td>Latin America</td>
<td>enrofloxacin, danofloxacin, norfloxacin</td>
</tr>
<tr>
<td>Australia</td>
<td>none</td>
</tr>
</tbody>
</table>

Use of Quinolones in Food Animals and Potential Impact on Human Health 99
be 50 tonnes, predominantly in the EU, USA, Japan and South Korea. Fluoroquinolone consumption by companion animals is much less and does not exceed 4 tonnes annually. Because of the low prices for generic fluoroquinolones, the amount of active ingredient of these products is estimated to be roughly 70 tonnes, which results in a total amount of approximately 120 tonnes active ingredient of fluoroquinolones (old generation quinolones excluded) for the above survey.

It would be of interest, of course, to have a worldwide survey but reliable data are not available from all countries and geographic locations, as elucidated below. Use in many third-world countries is especially difficult to determine because in most cases national trade associations do not exist to provide the sales figures of antibiotics in contrast to the industrialised countries. Moreover, many small companies exist which are reluctant to provide data. These markets can be generally characterised as follows: patent protection is poor, or non-existent. Consequently, relatively inexpensive (generic) quinolones are available on the market because the usually local, small companies do not incur the high costs of research, development and overhead. Moreover, registration requirements, if any, are minimal, as holds true for control on distribution of drugs. Furthermore, many kinds of low-quality counterfeit quinolones are in the market. Counterfeit drugs are predominantly produced locally but some are manufactured in industrialised countries as well. Antibacterial drugs are readily available over-the-counter, which allows farmers to treat the animals themselves, possibly with the wrong medicine or with the wrong dosages or duration. In sum, it is likely that worldwide sales in value of fluoroquinolones for food-producing animals considerably exceed the figures presented above, maybe by 50% or more. Given the low price of these generics, the sales in quantities of active ingredients would be an even much higher percentage.

Differences between industrialised and developing countries, with regard to quinolone application in animal medicine, become more clear when the use rate of quinolones is taken into consideration. Estimates from Western Europe indicate that the fluoroquinolones represent only a very small fraction (less than 1%) of the total consumption of therapeutic antimicrobials by food animals. We collected estimates for Denmark, Japan, Sweden, The Netherlands and United Kingdom and assessed that in these five countries sales of fluoroquinolones in weight account for an average of 0.43% of all antimicrobials used for therapy in veterinary medicine. The expense of these drugs prohibits their widespread use. In contrast, use rate of quinolones in countries with poor patent protection (e.g., Asia, Latin America) is markedly higher. It is assumed that in Spain the majority of the chickens are treated with (cheap) generic fluoroquinolones. High use rates in some countries are due to low prices of generic quinolones, over-the-counter practices (no prescription required), a less restricted licensing policy, a lower level of good management and hygiene, a limited cooperation with diagnostic laboratories as well as less access to accurate information and educational programmes.

It is of interest to make a comparison with fluoroquinolones used in human medicine. Generally, fluoroquinolones approved for people are analogues of veterinary quinolones.
and there are no basic differences among them, for instance regarding chemical structure, mode of action or antibacterial spectrum. Antimicrobial resistance to one fluoroquinolone confers reduced susceptibility or resistance to the entire class of fluoroquinolone agents. For specific reasons (e.g., side effects, toxicity, kinetics, diseases) a given quinolone might be more suitable for application in animals or humans. There are, however, major differences in the number of fluoroquinolones used in human medicine and the quantities produced. Currently approximately 15 fluoroquinolones are approved, the first one (norfloxacin) was authorised in 1983. All are commercially available in one (some of them) or many countries (the majority). Additional ones are still in the development pipeline. The sales of fluoroquinolones for use in humans in the above survey of more than 30 countries are estimated to be at least 3.0 billion dollars (year 1997) which may correspond to approximately 800 tonnes active ingredient. These figures exceed those for animal health by far. The same uncontrolled manufacturing and distribution of cheap generics that exist for animal fluoroquinolones in some developing countries also occurs with the human quinolones. Therefore, worldwide sales of human quinolones, particularly in weight, are estimated to be considerably higher.

What about future use of fluoroquinolones? It is generally accepted that there is a need for effective antimicrobial agents for treatment of infections in food animals. Diseased animals have the rights to be treated by adequate, state-of-the-art medicines. It is further believed that veterinarians that treat diseased farm animals should have access to different classes of antimicrobial agents, including innovative classes of agents such as fluoroquinolones or cephalosporins. This starting point is true now and for the future although improved management and hygiene as well as increased application of preventive medicine such as vaccination, immunomodulation or competitive exclusion should create opportunities for a reduction of antibiotic usage.

It is imperative, however, that adequate public health safeguards are in place in order to minimise any meaningful emergence and transfer of antimicrobial-resistant pathogens to humans through the food supply. It must be the objective of all involved to achieve a minimal transmission of bacteria—both susceptible and resistant—to humans. Efforts to this effect must include the support of, e.g., the animal health industry, the veterinary and medical profession, food processing industry, governmental authorities and international organisations such as WHO, FAO or OIE. The situation demands that regulations are enforced internationally because drug-resistant organisms do not respect national boundaries. The representative of the world’s animal health industry, COMISA, is willing to do its part and will make firm proposals on how the potential risk of transfer can be reduced. We actively support the implementation of measures such as:

- Strict requirements for licensing of fluoroquinolones for disease therapy which include veterinary prescription.
- Fluoroquinolones should be only used under the close supervision of a licensed veterinary surgeon. A diagnosis should be based, wherever possible, on bacterial culture and sensitivity testing.
• Only authorised fluoroquinolones are to be used, in strict accordance with the terms of their marketing authorisations and extralabel use in food animals should be restricted.

• Registration of fluoroquinolones neither permits prophylactic treatment of healthy animals nor usage as performance enhancer.

• Vigilant susceptibility surveillance over time in a coordinated manner should be carried out. This ensures that the potential risk of resistance can be identified and steps can be taken to manage the situation at an early stage.

• Post-approval surveillance of antibiotic use should be conducted.

• Education and training programmes for prescribers and users as well as marketers should be encouraged; direct advertising of fluoroquinolones to farmers should be discouraged.

• Research on the emergence and dissemination of antimicrobial resistance should be promoted. For instance, better epidemiological evidence is needed on how resistance develops and how resistance is spread within and between species.

If above steps are implemented, if not already done, and consistently complied—both in industrialised and developing countries—we are fully confident that the usefulness of this valuable class of antibiotics will be preserved for human and animal medicine. The fluoroquinolones offer significant benefits in antimicrobial therapy and animal welfare and we believe, provided that above guidelines are followed, that the prudent and responsible usage of fluoroquinolones in animals continues to be justified.
10 The Authorisation of Antimicrobials in the EU for Veterinary Use

Peter GH Jones

Introduction

Antimicrobials for use in animals broadly fall into two categories within the European Union. The first group are those administered at sub-therapeutic levels as growth promoters in food animals and are authorised according to the requirements laid down in Community legislation as presented in Council Directive 70/524/EEC (1). The second group, which is the subject of this paper, comprise those medicinal products used in animals for therapy against disease. In the European Union the measures in place to control authorisation of veterinary medicines including antimicrobials are contained in Council Directive 81/851/EEC (2), and the actual requirements established for testing veterinary medicines are detailed in Council Directive 81/852/EEC (3).

The European Community has undertaken significant steps to harmonise the requirements for authorisation of antimicrobials to treat disease in animals, so that the requirements for approval of such medicinal products, based on the criteria of Quality, Safety and Efficacy are the same throughout the 15 Member States of the Union.

The overriding principle which prevails in the regulatory process for medicines in animals is one of precaution both to the target animal and primarily in the case of food animals, the consumer. In this context therefore, the evaluation of data submitted in support of an application to authorise a medicinal product and the ultimate recommendations made by the authorities for its use, are reliant on the risk assessment approach. Such an approach balances the benefits of providing medicines to ensure the health and welfare of animals being treated, against the potential risks, in the case of food animals, to the consumer whose safety is paramount. However, such an assessment is heavily dependent on knowing the magnitude of the risk. The ongoing debate about the potential role of antimicrobial use in veterinary medicine in the development of resistance to such products in man is regrettably often based on speculation, so that any assessment on the magnitude of this risk is rarely based on scientific fact.

Regulatory Controls

Whilst the approximation of the laws of Member States relating to veterinary medicines began in the early eighties, it was in January 1995 that the European Community witnessed a major advance in the licensing of veterinary medicines with the opening of the European Medicines Evaluation Agency (EMEA) in London, accompanied by the introduction of two new systems of authorisation.
The centralised procedure is the first of these new procedures co-ordinated by the EMEA and enshrined in Community law under Council Regulation (EEC) 2309/93 (4). It is compulsory for medicinal products derived from biotechnology and for innovative yield enhancers. It is also available at the discretion of applicants for other new products classed as innovative (e.g. new claim for which no other medicine exists, new delivery system and/or a new drug entity etc). Applications are submitted directly to the Agency in London and the evaluation is undertaken by the Committee for Veterinary Medicinal Products (CVMP) and its team of expert advisors. At the conclusion of the scientific evaluation undertaken in 210 days within the EMEA, the opinion of the CVMP is transmitted to the European Commission to be transformed in a further 90 days into a single marketing authorisation valid throughout the 15 Member States of the European Union.

In support of Community legislation the CVMP has elaborated an extensive series of guidelines on the testing of veterinary medicinal products in the Community covering a range of subjects extending from topics as diverse as antimicrobials for general veterinary use, to bioequivalence, to the testing of antiparasitic products and to the control of immunological veterinary medicinal products. These guidelines are published in Volume VI of the Rules Governing Veterinary Medicinal Products in the European Union (5).

Additionally, since 1 January 1992 it is a requirement within the EU under Council Regulation (EEC) 2377/90 (6) that any new medicinal product intended for use in food animals shall be assigned a set of Maximum Residue Limits (MRLs), which set a threshold above which residues will not be tolerated in the food and food products derived from that animal in order to prevent risk of exposure to the consumer. The assessment of MRLs for antimicrobial substances is reviewed in further detail below.

**Specific Data Requirements for Antimicrobials**

To date the therapeutic use of antibiotics in food producing animals has not been thought to result in a significant risk to consumers because the dose levels of the antimicrobials used are high and are administered for relatively short periods. Such short-term therapy has not been considered to result in extensive selection of resistant microorganisms and has allowed recovery of the endogenous microflora. Consequently the extent to which data have
been required to establish whether the product is capable of selecting resistance factors less than those generally required to support sub-therapeutic, feed additive use.

A) Resistance Development in Animals

Chapter 1c of Part 4 of the Annex to Council Directive 81/852/EEC as amended requires that data on the emergence of resistant organisms are necessary, and further elaboration is given in the various guidelines on the use of antimicrobials in veterinary clinical medicine. The key requirements in these guidelines is that the applicant must provide data giving details of which microorganisms are fully sensitive and resistant, based on tests using a minimum of 10 strains (epidemiologically unrelated) of each species against which an effect is claimed. Furthermore the test must indicate to what extent resistance can be expected to develop, how rapidly and under what conditions. It is seen to be imperative that the applicant provides baseline bacterial susceptibility data, not only to support efficacy claims but also to support future monitoring under field use where this is deemed necessary in post marketing surveillance activities. The occurrence of cross-resistance should be discussed fully and the potential significance of plasma protein binding of the drug should be stated as follows.

i) The sensitive microorganisms should be listed in increased order of MIC if there are clinical efficacy data to support clinical use.

ii) When the difference between MIC and MBC (Minimum Bactericidal Concentration) values is very small or large this should be stated.

iii) Care must be taken to distinguish between in-vitro and in-vivo data and clinical correlation must be provided to support claims based on in-vitro data.

iv) If synergism or antagonism has been found with other active ingredients and been demonstrated to be clinically significant, this should be stated.

Extensive pharmacokinetic data are also required to demonstrate clearly that the selection of the formulation and the dose recommended provides for optimal levels of drug to be made available at the anticipated site of action against the bacteria for which a claim is being made.

It is implicit in the guidance notes that such pharmacokinetic data are intended to ensure that the best dosage form has been selected and the appropriate conditions of use can be set. When such products are being administered to large groups of animals on a flock or herd basis, the regulatory process will try and ensure that therapy via the feed or drinking water is adequately managed to guarantee accurate treatment regime. Throughout the European Union the use of antimicrobials are subject to use on the prescription of a veterinarian only, to ensure no indiscriminate use by lay persons. Such prescription therapy has to be for animals under the care of the veterinarian and should therefore not be prescribed “at a distance” without prior clinical examination.
B) Microbiological Risk to the Consumer due to the Presence of Residues

As well as the requirement to establish the sensitivity of various organisms there are extensive measures in force, as with all veterinary medicines, to establish maximum residue limits for those products destined for use in animals from which food and food products are derived for human consumption. The data requirements to establish an MRL in the European Union are set out in Annex V of Council Regulation (EEC) 2377/90 and guidance on preparing the application is presented in Volume VI of the Rules Governing Veterinary Medicinal Products in the European Union. In the case of antimicrobials, microbiological endpoints as well as toxicological end-points must be determined in the elaboration of the MRLs. This approach to setting MRLs is very similar to that used by the FAO/WHO Expert Committee on Food Additives (JECFA) and commonly results in identical MRLs being determined by the EU and JECFA; where differences arise this is often due to different data being available to the two organisations at the time of assessment, which can differ by a matter of years.

Conventionally, the safety of the active substance is established by the calculation of an Acceptable Daily Intake (ADI) from the No Observed Effect Level (NOEL) derived from a battery of pharmacotoxicological studies divided by an appropriate safety factor to account for the extrapolation from animal studies to man and multiplied by 60, determined to be the average weight in kilograms of the European consumer. The safety factors may vary from a factor of 100, a multiple of 10 for greater sensitivity of man to animals and a multiple of 10 to account for the range of sensitivity in man, to as high as 2000 depending on the toxicological characteristics of the compound.

\[
ADI = \frac{NOEL \times 60}{Safety\ Factor}\]

Where antimicrobials are concerned, the setting of an MRL also requires that the potential effects of antibiotic residues on human gut flora are examined and the same objective scientific strategy has to be followed in evaluating the risk of microbiological residues as is used to explore potential toxicity (Boisseau) (7). Three possible effects must be considered as resulting from exposure to antimicrobial residues.

i) Specific infection by the dominance of a known enteropathogen e.g. Salmonella.

ii) An increase in the number of potential pathogens e.g. enterobacteriaceae or of drug resistant bacteria, which can be particularly dangerous in immunocompromised patients.

iii) Damaging changes to the commensal bacteria in the intestinal tract.

A combination of any of these 3 phenomena resulting from exposure to microbiological residues in the consumer can potentially harm the impressive barrier effect provided by the endogenous population of the intestinal tract as a protection against invasion of pathogenic bacteria and subsequent disease.

Unfortunately however, the actual conduct of the tests to determine microbiological end-points is difficult. There is little agreement on how to conduct such studies or on
the interpretation of their results. Three types of studies can be conducted:

- in human volunteers
- in animals
- in in-vitro systems

All these types of study are considered by many to be inadequate for the task and there is some doubt about their validity, with concerns about whether the results from such experiments really can provide a sound scientific basis for concluding whether a certain substance or other when present as residues in the tissue or food products in treated animals, present a real risk to the consumer. However, although there is insufficient evidence to demonstrate whether antibiotic residues present a risk to the consumer it is probably better to err on the side of caution and demonstrate as far as possible that they are unlikely at levels below the established MRLs to cause perturbations in the human gut flora (Woodward) (8).

Once the safety of the product has been assessed by the establishment of the ADI, residue studies which must also be conducted provide information as to the metabolic profile of the active substance. The marker residue must be established in the target tissues and its decline measured over time. Based on a set of consumption figures for a market basket of European foodstuffs set arbitrarily high, the MRLs can be established. Once the risk of exposure is assessed, that risk is then managed by the setting of a suitable withdrawal period, which is the necessary time between treatment and slaughter for the residue levels to decline below the MRLs.

**Recommendations for Fluoroquinolones**

Currently there are no specific requirements for regulation and authorisation of any specific class of antibiotics marketed in the European Union. It may be useful to review the experience gained in the authorisation of two of these products to date and speculate as to what further controls and recommendations may be necessary in future.

As with all veterinary medicinal products licensed under Council Directive 81/851/EEC, Article 5a requires that a standard set of particulars should be contained for each product in a Summary of Product Characteristics (SPC). For centrally approved products there is a common SPC for the Community authorisation which is common for all Member States and identical in its content except for the different languages in which it is available. A harmonised approach also exists for a common SPC for a decentralised procedure the contents of which are agreed during the mutual recognition process. However, for those products authorised under the old national systems by the individual Member States each competent authority (licensing body) will have agreed its own format and content with the applicant concerned.

For the product enrofloxacin as an oral solution in poultry the advice given in the SPC regarding the use and recommendations to be followed by the veterinarian in the context of a judicious approach to when and where to apply the product differs considerably in different countries. In one Member State the advice given states that use of
the product is recommended where clinical experience supported where possible by sensitivity testing of the causal organism indicates enrofloxacin as the drug of choice. In other countries the recommendations for product use are directed at situations where the disease is caused by organisms sensitive to the product whilst in others no advice on precautionary measures for careful use of the product is given at all. Now that the two new systems of authorisation are in place in the European Union, it is to be expected that equivocal advice such as this given in different Member States for the same product, in the same therapeutic form, for use in the same species against the same disease entity will cease to exist. Nevertheless the pharmaceutical industry should also put its house in order to standardise its approach in such cases and make the necessary changes in the product literature to ensure equivalent recommendations consistent with good clinical practice and respect for the careful use of such products. This is essential if success is to be achieved in reducing the emergence of resistance and prolonging product life-span. Not to do so lays the industry open to criticism that the sponsor companies involved are driven by a commercial expediency to maximise product sales in certain markets, at the expense of any consideration regarding controlled and sensible use of such products in veterinary medicine.

The CVMP last year gave a positive opinion in respect of a new fluoroquinolone difloxacin for use as an oral solution in poultry through the centralised procedure (9). The Committee was cognisant of increasing concerns about the potential for resistance development of this important class of antimicrobials and considered in detail a report on resistance monitoring with difloxacin by the Regional Animal Health Service in The Netherlands of 971 bacterial isolates from diseased poultry from 1993-1996 i.e. prior to its authorisation. The data included results from monitoring in poultry comprising 75% chickens and 25% turkeys and showed low levels of resistance (5-6%) of resistance to E. coli, Salmonella spp and Pasteurella multocida isolates. It was noted that no significant change in the resistance levels could be seen during the years of surveillance, despite the introduction and widespread use of quinolones in Dutch veterinary practice since the mid-eighties. The market authorisation holder has agreed to continue the resistance monitoring programme with an extension to other European countries now that the product is commercialised and data will be submitted to the CVMP for periodic review. In this instance the Committee was mindful of its responsibilities in assessing the risk and it was only after careful consideration of this data that the CVMP was able to conclude whether the benefits resulting from the authorisation of this product outweighed such risks to justify a positive opinion.

In its deliberations on the type of advice to be given in the SPC on when to prescribe this product, the Committee considered at length whether to restrict its use to that of a second choice antibiotic; such use would be envisaged in situations where use of a more conventional antimicrobial had failed. The Committee recognised however that clinical judgement in a serious disease situation in commercial poultry houses demands that treatment of such conditions as are indicated for use of these products be required quickly to avoid large-scale mortality and extensive spread of the disease. Consequently
the CVMP recommended that use of the product be restricted to situations where prior sensitivity testing has demonstrated on particular farms that treatment with established antimicrobials would be unlikely to succeed. Nevertheless it is recognised that results of prior sensitivity testing in a clinical disease situation may become available too late in a situation to effectively treat and control the infection. It is to be hoped that increased attention can be given by clinicians to continually monitor sensitivity patterns in flocks and herds of animals under their care and supervision, so that better therapeutic regimes can be devised associated with cautious use of the new classes of antimicrobials to reduce development of resistance.

Current and Future Initiatives by CVMP

In July 1997 the CVMP adopted a new guidance note on the conduct of pharmacovigilance in the veterinary sector (10). Amid a certain amount of criticism the Committee extended what had previously been considered the limits defining pharmacovigilance from Serious Adverse Reaction (SAR) reporting to some new parameters. Probably the most important of these is that companies must in future report available evidence of emerging trends of resistance from epidemiological surveillance following the authorisation of antimicrobials which they commercialise. The CVMP in developing these guidelines considers it essential that means should be introduced to monitor emerging trends of resistance so that the necessary precautions can be applied to optimise the use of these products and to reduce the extent of resistance development.

In addition the CVMP undertook a major new initiative to conduct an in-depth study of the resistance to antimicrobials in the veterinary sector in the European Union. An ad-hoc working party of the Committee has been established consisting of independent experts from the majority of the EU Member States recognising that too long action has been called for and recommendations have been made in the context of antimicrobial resistance with insufficient knowledge of the extent of the problem. The mandate of this group is to conduct an in-depth scientific assessment of the incidence of antimicrobial resistance among bacteria isolated from animals, based on an epidemiological analysis of the current extent of the problem. Such an analysis shall broadly consider use patterns of antibiotics in veterinary medicine (broad and narrow spectrum), the rate, extent and type of resistance development, and the relationship between them all. Consideration should be given as to what degree resistance will affect the efficacy of antibiotics in animals in the future. Furthermore the group will also assess the risk of transfer of resistance from animal bacteria to man and to what degree this phenomenon might occur. The group should distinguish between genetic and transferable resistance and the impact on the success with which antibiotics are used in treating animals. The remit of the scientific group of experts will be confined to the use of therapeutic antibiotics authorised under the terms of Council Directive 81/851 (EEC) only. The group should report its findings to the CVMP by 31 December 1998.

Once this first phase is completed the CVMP will consider the findings of the scientific experts and consider ways of managing the
problem of resistance in animals with a view to reducing the risk of transfer to man, if it is shown by the experts to exist. At the same time the CVMP will take account of the impact of a potential reduction in the availability of new antibiotics and the impact of such an outcome on animal health and welfare and the benefits currently achieved by their use in treatment of disease. The successful treatment and control of disease in animals also has a significant and beneficial effect on public health itself.

One of the ways of managing the problem will be to develop a protocol for conducting the epidemiomosurveillance programme for monitoring resistance development in the post marketing phase after authorisation of new antimicrobials.

Discussion

Within the European Union the approximation of laws of the Member States provides for a harmonised approach to the authorisation of veterinary medicines including antimicrobials. The measures in force provide for pre-authorisation sensitivity testing of the antibiotic to demonstrate that it is the most appropriate drug for the treatment intended and as a basis for resistance monitoring. Nevertheless there is still a certain lack of consistency in some Member States as to the information on pathogen susceptibility on product labelling. In addition there needs to be further consideration in the case of the fluoroquinolones as to the appropriate degree of restrictive labelling that may be required for these products. The result of the CVMP ad-hoc working group’s investigations will prove invaluable in addressing this issue.

In addition the requirements in place for the determination of microbiological end-points in setting Acceptable Daily Intake (ADI) to establish MRLs appear more than adequate to satisfy the principle of precaution in protecting the consumer from residues of antimicrobials, that may be detrimental in contributing to the resistant pool of organisms in man which may comprise the efficacy of related and other antibiotics in the treatment of human infectious diseases. However, over-restrictive requirements which are not grounded in scientific fact can lead to the establishment of longer withdrawal periods (the time between treatment and slaughter) than are necessary which unfairly penalise an already contracting animal health industry. The danger of such a scenario would be further restriction on the provision of new medicinal products for animals, which they require just as much as their human counterparts if their health and welfare are to be guaranteed which is certainly the case in our society today. A restriction on the use of new and innovative products, such as a ban on fluoroquinolones, could lead to illegal use of these compounds which would be to no-one’s benefit as the situation in the EU with hormonal products has demonstrated.

The veterinary surgeon is at the forefront of the use of antibiotics in veterinary medicine and bears enormous responsibility to ensure they are not misused. These medicinal products must clearly remain on a prescription only basis for treatment of animals directly under his or her care. There can be little justification for general prophylactic use and certainly not for extra-label use with all the attendant risks that the latter may result in.
Continuing professional development is widely practised by the veterinary profession in a number of countries. Part of such a commitment must be for the profession to ensure that its members are aware of the responsibilities they have. The majority do, but there are those undoubtedly who do not fully utilise their professional judgement in taking the cautious approach that is now recognised as being essential for the use of antibiotics especially the newer ones including fluoroquinolones. A routine decision to use these newest and most valuable antibiotics as a first choice and without prior knowledge of susceptibility patterns on farm must be avoided and professional associations must take their responsibilities seriously in this regard. The British Veterinary Association (BVA) in its recent evidence to the UK Advisory Committee on the Microbiological Safety on Food has recommended appropriate codes of practice for all its members to encourage responsible veterinary prescribing and use of antimicrobials (11). The BVA has committed to take the lead in informing the profession and providing:

(a) guidance on approaches to the treatment of individual species and, of key importance

(b) guidance on the decision trail prior to the administration of antibiotics—narrow spectrum to broad spectrum.

Other professional associations need to follow similar procedures where they have not done so already.

In a recent editorial in the Veterinary Record (12) the BVA commented with regret that it is now nearly thirty years since the Swann report called for further research into the problem of resistance, and that as then, there remains today a dearth of evidence concerning the risk to man from the use of antimicrobials in animals. This in turn has led to a polarisation of opinion and increases the force with which views are expressed.

An example of such polarisation was illustrated recently in correspondence by Threlfall et al. (13) where it was suggested that the emergence and spread of isolates of multi-resistant Salmonella typhimurium (DT) 104 in the UK with reduced sensitivity to ciprofloxacin followed the licensing of the related fluoroquinolone enrofloxacin for veterinary use in late 1993. Such a claim as presented in this particular instance appears to have been unsubstantiated and based on a temporal relationship only, without evidence of scientific data, and indeed failed to consider the implications of inappropriate use of fluoroquinolones in man as a contributory factor. Not surprisingly this resulted in a hostile response from the veterinary profession (14) who understandably objected to unjustified and seemingly unproven claims that the fault lay solely with the use of these products in animals. Progress will not be made by a continuation of learned parties trading accusations and counteraccusations with each other. Both the medical and veterinary professions must work in closer collaboration with one another to address this complex issue because neither wants to see a reduction in the efficacy of these compounds. The problem has to be quantified taking into account both veterinary and human use and a collaborative approach is essential.

In its recently published report on Resistance to Antibiotics and other Antimicrobial Agents (15) the United Kingdom House
of Lords Select Committee on Science and Technology has made a number of critically important and worthy recommendations placing responsibility on both the medical and veterinary sectors. It calls for an extensive education programme for members of the medical profession for prudent use of these substances. It also calls for greater attention to be focussed on the use of food additive antimicrobial growth promoters without necessitating the implementation of an outright ban. Of particular interest to this meeting is its recommendation for extreme economy of use for fluoroquinolones in veterinary practice, notwithstanding the right of large and companion animals to receive such agents on an individual basis for short term therapy but cautioning against mass treatment of herds of pigs and flocks of poultry. The CVMP will, I believe, certainly consider this report and its findings in its own deliberations on the subject.

The regulator in veterinary medicines meanwhile is caught in the middle and needs to make decisions on the rights and wrongs of authorising these medicines recognising on the one hand the health needs of animals; failing to do so leads to diseases which are a further threat to public health, and safety to consumers on the other. To successfully complete the risk assessment implicit in the European requirements for licensing such antibiotics the magnitude of the risk must be established first before the risk, if it shows to exist can be managed. To assess the magnitude is therefore clearly the challenge, and the goal must be the provision of extensive data through monitoring programmes. Until that information is available no one party can lay the blame at the other’s door. Failure in 1998 to have conducted the research that the Swann Committee called for nearly 30 years ago in 1969 is an abrogation of responsibility for all concerned, the medical profession, the veterinary profession, industry and regulators and we do ourselves a disservice to make any attempt at managing the problem before that essential element in this equation is provided – sound scientific fact.

References


11. Personal communication.


11 QUINOLONE LICENSING FOR USE IN FOOD ANIMALS AND RELATED PROBLEMS FACING REGULATORS IN THE USA

Stephen F Sundlof and Sharon R Thompson

The U.S. Food and Drug Administration's (FDA's) Center for Veterinary Medicine (CVM) is concerned that the use of therapeutic antimicrobial drugs in food animals will create antimicrobial drug resistance that could contribute to drug-resistant human pathogen bacteria. CVM is discussing our concerns about antimicrobial usage with other parts of FDA and the U.S. Centers for Disease Control and Prevention (CDC).

CVM agrees with the following points:

1. There is a legitimate need for both older and newer antimicrobial drugs in animal agriculture.

2. The bulk of *Salmonella, E. coli O157*, and *Campylobacter* infections in humans in the U.S. originate from food of animal origin.

3. The use of antimicrobials in animals will cause resistance to develop, and there is a potential that resistant *Salmonella, E. coli*, and *Campylobacter* will be transferred to humans through food.

**Status of Antimicrobials**

For several years, CVM has approved new antimicrobials for use in animals for therapeutic purposes as prescription-only products. This prescription-only policy is based on CVM’s desire to assure the proper use of antimicrobials though precise diagnosis and correct treatment of disease to minimize animal suffering and to avoid unsafe residues. Antimicrobial products for use in animals have to meet FDA’s stringent standards for safety, efficacy and quality to be approved in the U.S.

When products are intended for use in food producing animals, safety considerations include the evaluation of data to ensure that residues in food derived from treated animals are safe for human consumption. In the past, microbiological safety studies were only required for antimicrobials to be used in feed for more than 14 days. These studies examined resistance patterns and pathogen load.

Because of CVM’s current concern over antimicrobial resistance, CVM now believes that the safety assessment of antimicrobials must include evaluation of resistance concerns with the conduct of pre-approval studies and post-approval monitoring programs (PAMPS). However, CVM will consider waiving such requirements for products of low public health concern. The details of the post-approval programs will
vary on a product specific basis. Product specific PAMPs may be used to supplement data collected through the National Antimicrobial Resistance Monitoring System created in the U.S. in January, 1996. This program is a collaboration among FDA, CDC, and USDA and monitors shifts in antimicrobial susceptibilities of 17 antimicrobial drugs in zoonotic enteric organisms from both veterinary and human sources.

In granting approval for certain antimicrobials, CVM may also seek a commitment from the drug sponsor to voluntarily stop sale of their product if new scientific evidence is presented post approval, that demonstrates use of the product may present a risk to public health. This evidence may be collected either through a product specific PAMP, the general monitoring system, and/or from studies published in the scientific literature. This commitment is intended to supplement FDA’s current authority to remove products from the market once approved.

**Prudent Use**

CVM believes it is critical that prudent use of antimicrobials be emphasized in order to minimize the development of antimicrobial resistance and to ensure the continued efficacy and availability of antimicrobial products for use in food-producing animals. To promote this concept, CVM and CDC jointly facilitated a meeting on “Prudent Use” held May 6, 1998, in Rockville, Maryland.

The objective of the meeting was to develop a plan to promote the prudent use of therapeutic antimicrobials in veterinary medicine. The focus of the meeting was limited to therapeutic antimicrobials.

Key elements of prudent use that CVM believes should be addressed include:

1. Development of prudent use principles.
2. Therapeutically based antimicrobial use guidelines.
3. Recommendations on appropriate measures to reduce disease transmission.
4. Educational programs for prescribers and users of these drugs.

CVM defines “Prudent Use” of therapeutic antimicrobial agents as the...

“Use that maximizes therapeutic effect while minimizing the development of resistance.”

At that meeting, CVM solicited advice from the human medical community in the development of “Prudent Use” principles because their expertise about what has worked and not worked in human medicine will be useful. CVM will continue to draw upon the expertise of the human medical community as prudent use programs are developed within the veterinary community. The development of the Prudent Use principles will not be static. Instead, the process will likely demand continued attention. In fact, CVM likely will engage food animal producers on this issue at some time in the future.
Future Regulation of Antimicrobial Products

CVM views as its top priority the need to define scientifically based criteria and/or standards for the regulation of antimicrobial products. The Center expects that these efforts will help long-term to create a stable regulatory environment for these products.

The following are some of the issues on the regulation of antimicrobial products that will need to be addressed by CVM:

1. Does CVM have the necessary tools to remove from the market products that present a public health concern AFTER approval?

2. Should CVM use the same criteria for assessing safety for therapeutic and non-therapeutic products?

3. What criteria should be used to define whether a particular antimicrobial product will need to undergo pre-approval studies to evaluate resistance concerns and should have a post-approval monitoring component to continue this evaluation?

4. Is CVM’s current definition of therapeutic appropriate for the current regulatory environment?

5. Should the establishment of withdrawal periods include evaluation of pathogen load and resistance patterns?

6. Are there additional research areas that need to be addressed?

Approval of Quinolones

CVM’s view is that approval of quinolones for therapeutic use in animal agriculture is appropriate under the following conditions:

1. The product meets all FDA requirements for safety, efficacy, and quality.

2. A system is used to ensure that once approved, products are used in accordance with labeled indications and prudent use principles.

3. There is a monitoring system to track changes in resistance.

4. Corrective action, including product withdrawal as a last resort, can be taken if scientific evidence is presented post approval which demonstrates continued use of the product may present a public health concern.
12 **Overview of Fluoroquinolone Use in US Poultry Production**

Mark C Bland

There are two fluoroquinolone (FQ) products approved for use in poultry in the United States (U.S.). Sarafloxacine hydrochloride (Saraflo 10% water soluble solution) was first approved in September of 1995. An injectable form of Saraflo (50 µg/ml) for use in day-old and in ova injection was approved in Jan. of 1997. The injectable application is only approved in the U.S. Enrofloxacine (Baytril) is a 3.23% concentrate antimicrobial solution approved for use in poultry in October of 1996.

Sarafloxacine is indicated for the control of mortality associated with *E. coli* organisms susceptible to sarafloxacine in broiler chickens and growing turkeys. The product is administered in the water at 20-40 ppm (broiler chickens) and 30-50 ppm (growing turkeys) for 5 consecutive days. The injectable form of sarafloxacine is administered at 0.1 mg, SQ in 0.2 ml (day old hatching chicks) and 0.1 mg in 0.05 ml (in ova at transfer).

Enrofloxacine is indicated in chickens for the control of mortality associated with *E. coli* susceptible to enrofloxacine. In turkeys it is indicated for the control of mortality associated with *E. coli* and *P. multocida* (fowl cholera) susceptible to enrofloxacine. The product is dispensed at 25-50 ppm in solution for three-seven days.

**Use Guidelines**

The use of FQ in food animals is restricted to poultry only in the U.S. Extra label use in food animals is strictly prohibited (Federal Register, May 22, 1997). FQ can only be used under prescription from a licensed veterinarian who is overseeing the general health of the flock. Bacterial cultures and sensitivities are routinely performed at diagnostic laboratories. This provides important information for veterinarians to determine a course of action. Neither product is labeled for use in laying hens producing eggs for human consumption. Due to the pharmacokinetic differences of the two FQ the withdraw time for enrofloxacine is two days and sarafloxacine is zero days.

**Disease Agents Related to FQ Use in Poultry**

Chickens: Airsacculitis caused by *E. coli* is a common sequela to a number of immunosuppressive viral infections (Herpes, Birna, Retro, CAA), respiratory infections (NDV, IBV, and mycoplasma), and environmental/management conditions (failed vaccination programs, air and litter quality etc.). In broilers this happens around one to five days of age (mycoplasma related) and four to seven weeks of age.
Turkeys: E. coli septicaemia and Airsacculitis infections are secondary to a host of viral, bacterial, environmental, and management conditions. These include Hemorrhagic enteritis (HE), Pneumovirus, Corona virus associated with PEMS (poul teratitis morality syndrome) Ornithobacterium Rhinotracheale (ORT) B. avium and management (air/litter quality etc.).

**FQ Usage in the Poultry Industry**

The following three tables provide information on FQ usage in the U.S. The data was provided by U.S. poultry integrators who purchased the products.

There are some reasons for the differences in the numbers between broilers and turkey. Turkeys are marketed at a much older age (40-48 days for broilers vs. 100-140 days for turkey). Also the Turkey Industry has been experiencing a number of pneumonia outbreaks in the Midwest and PEMS in the southeast.

**Strategies for Dealing with Resistance Issue**

There are a number of established programs in place at the government, industry and farm level that either directly or indirectly relate to the subject of drug resistance in zoonotic bacteria. Post approval monitoring

---

**Table 1**  
**Fluoroquinolone Usage in the U.S. Broiler Industry (1997)**

- 8.3 billion broilers produced  
- approximately 3% of the broilers became ill  
- approximately 1.2% received antibiotic therapy  
- approximately 0.46% received FQ in 1997 at an average age of five weeks

---

**Table 2**  
**Fluoroquinolone Usage in the U.S. Turkey Industry (1997)**

- 300 million turkey were produced  
- approximately 23% of the turkeys became ill  
- approximately 16.2% received antibiotic therapy  
- approximately 4.2 - 8.7% received FQ in 1997 at a treatment age and between 3 and 11 weeks

---

**Table 3**  
**Fluoroquinolone Usage Relative to the Meat Consumption**

<table>
<thead>
<tr>
<th>Species</th>
<th>Lbs. produced</th>
<th>Per capita consumption</th>
<th>Treated Lbs. per Capita</th>
<th>% treated of total meat consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>41.0 billion</td>
<td>64.0 lbs.</td>
<td>0.29 lbs.</td>
<td>0.12 %</td>
</tr>
<tr>
<td>Turkey</td>
<td>7.2 billion</td>
<td>17 lbs.</td>
<td>1.02 lbs.</td>
<td>0.43 %</td>
</tr>
</tbody>
</table>

120  Use of Quinolones in Food Animals and Potential Impact on Human Health
is currently being done by both manufactures of FQ. In addition, the poultry industry through diagnostic labs, culture and run sensitivity testing on bacteria on a routine basis.

The poultry industry has developed and implemented programs such as Best Management Practices and Quality Assurance Plans. The goal of these strategies is to produce the highest quality poultry that includes the safety of poultry and poultry products. The main focus of these plans is to reduce and to eliminate the introduction of bacteria to poultry on the farm. This is accomplished through biosecurity programs at the farm, hatchery and feed mill, cleaning and disinfection procedures, using integrated pest management (IPM), the use of Normal Avian Gut Flora (NAGF), probiotics and competitive exclusion products and the use of effective vaccines and vaccine programs. All of which will help reduce the opportunity of drug resistant bacteria from developing in food animals.

Preventative medicine is the goal of every poultry producer and veterinarian flock health professional. It only makes common sense to be prudent with the use of antibiotics and stress proper management techniques to provide for the safety of animals and humans alike.
13 Quinolone Usage in Poultry Medication in Europe

Paul F McMullin

Summary

A range of quinolone-based antimicrobials are licensed for use in poultry not laying eggs for human consumption in many European countries. In the UK only two, third-generation compounds are licensed for poultry, one of these recently licensed through a Europe-wide approval mechanism. Although indicated for the treatment of pasteurellosis and colisepticemia many quinolones are limited in their usage in Europe to high-value or very young stock or unusually severe disease because of their high cost compared to other antimicrobials. They are used to prevent suffering and mortality associated with these diseases and may play a part in the eradication of certain Mycoplasma and Salmonella infections from poultry stocks. Vaccination against primary viral pathogens and eradication of chronic infections should help reduce the need for all types of antimicrobial medication including the quinolones. All therapeutic antimicrobials are only made available under veterinary prescription in Europe. The patterns of quinolone usage and resistance in E. coli isolates in this practice are low and stable.

Introduction

Antimicrobials of the quinolone group have been available in some European countries for over 20 years (1). The discovery and development of third-generation fluoroquinolones (2) with a broader spectrum of efficacy led to interest in their use for the treatment and control of a number of important diseases of poultry (3).

Products licensed in Europe

Table 1 lists some quinolone antimicrobials licensed in some European countries for use in poultry. The range of products licensed, and their date of introduction varies widely. For instance, in the UK, only 2 third-generation compounds are available, enrofloxacin introduced in 1993 and difloxacin in 1997. Other European countries have oxolinic acid licensed, sometimes in combination with other compounds, although we understand that maximum residue levels (MRL’s) have not been established for this compound and usage is minimal. The fluoroquinolones are broad-spectrum in activity and their use may be exemplified by the indications listed on the enrofloxacin data sheet for the U.K.:

“Indicated for use in Turkeys, Broilers, Broiler breeders, and replacement chickens for diseases of the respiratory and alimentary tract of bacterial or Mycoplasma origin (e.g. pasteurellosis, mycoplasmosis, colibacillosis, colisepticemia and salmonellosis) where clinical experience, supported
where possible by sensitivity testing of the causal organism, indicates enrofloxacin as the drug of choice (4). Data sheet indications for this and other quinolone products in various European countries are very similar to this, though they are usually expressed in a shorter form. Note that most such products specifically exclude their use in birds producing eggs for human consumption. One third-generation quinolone product based on difloxacin has recently been licensed through a centralised European procedure.

**Specific Applications**

**Therapy of Coli-septicaemia and Pasteurellosis**

*Pasteurella multocida* is capable of causing very high mortality in affected poultry. The occurrence of the disease can be difficult to predict and vaccinate for, and commercial vaccines sometimes fail to protect against variant challenge bacteria. Septicaemia caused by *E. coli* is a common sequel to a range of primary viral infections in poultry. Highly pathogenic strains of *E. coli* may be present without causing significant disease until such time as another pathogen depresses the immune system or damages a mucosal surface. Although daily mortality is usually much less than in pasteurellosis it may continue over an extended period. Neither the *E. coli* strains nor the *P. multocida* strains associated with these diseases are believed to pose any direct hazard to human health. The main advantage of the quinolones in the treatment of these diseases is their tendency to concentrate in tissues of the respiratory system. However they would not usually be considered to be the products of first choice, for, among other reasons, the fact that they are much more expensive than most other antimicrobials.

Figure 1 shows the relative cost per kg of bird medicated of a range of antimicrobials licensed for poultry in the UK. In markets without advanced and complicated medicines licensing systems, and with less regard for patent protection, it is likely that the differential in cost between quinolones and other compounds will be much less.

**Mycoplasma sp.**

Primary poultry breeders have made great progress in eliminating *Mycoplasma* infec-

<table>
<thead>
<tr>
<th>Active</th>
<th>Where</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difloxacin</td>
<td>Europe</td>
<td>Chickens (Broilers/Future breeders), Turkeys to 2 Kg</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>UK</td>
<td>Chickens, Turkeys, except layers</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>France</td>
<td>Chickens, Turkeys</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Italy</td>
<td>All except layers</td>
</tr>
<tr>
<td>Flumequine</td>
<td>France</td>
<td>Poultry</td>
</tr>
<tr>
<td>Flumequine</td>
<td>Italy</td>
<td>All</td>
</tr>
<tr>
<td>Flumequine</td>
<td>Spain</td>
<td>Broilers</td>
</tr>
<tr>
<td>Oxolinic Acid</td>
<td>France</td>
<td>Poultry (chickens)</td>
</tr>
<tr>
<td>Oxolinic Acid</td>
<td>Italy</td>
<td>Broiler</td>
</tr>
<tr>
<td>Oxolinic Acid</td>
<td>Spain</td>
<td>Birds</td>
</tr>
<tr>
<td>Oxolinic Acid + Colistin</td>
<td>Italy</td>
<td>All</td>
</tr>
</tbody>
</table>
tions of clinical importance from breeding stock. This brings direct economic benefits as well as reduced need to medicate progeny for *Mycoplasma* infection and secondary bacterial infections. Quinolones have at various times, been used both in the eradication procedures and in dealing with occasional break-downs in commercial breeding stock. This may involve periods of treatment of the parent stock and/or of the progeny. There should be a progressive reduction in the need for such medication, and, in any case other more specific compounds may be more effective.

**Salmonella sp.**

Control of Salmonella infections in commercial poultry is usually based on obtaining Salmonella-free breeding stock, effective decontamination of feed raw materials or finished feed, effective decontamination of farms, and bio-security measures to prevent re-introduction of infection on to farms. Antimicrobial medication plays only a small and steadily diminishing part in such control programmes. Quinolones and other antimicrobials have been used in dealing with Salmonella-infected breeding stock. Early trials of the treatment of the poultry-specific *Salmonella pullorum* in laying stock were not encouraging (5). Programmes in which medication is followed by administration of natural gut flora were subsequently developed in the Netherlands (6). For example:

1. Medicate for 5-10 days
2. If possible move to a clean environment during the period of medication
3. Administer a competitive exclusion product 2-4 days after the end of medication.

While the success of such a programme is likely to depend on a number of factors, it is generally recognised that it is unlikely to completely eliminate infection in the treated birds. It can, however eliminate transmission to the next generation (7). While it might be argued that such medication should not be permitted and that all infected flocks should be immediately slaughtered, there are risks inherent in this also. The loss of the eggs from the slaughtered flocks could force companies to keep other flocks beyond their normal slaughter age, reduce farm turnaround times and hence cause future disease problems. It might also result in importation of hatching eggs infected with the same or other *Salmonella* serotypes into the organisation and these might eventually result in an increased and/or different *Salmonella* challenge for other breeding flocks. The decision is not as simple as it might seem!

---

**Figure 1** UK approved antimicrobials relative cost/ kg medicated

---

*Use of Quinolones in Food Animals and Potential Impact on Human Health* 125
Prescription-only medication in poultry practice – the implications

Under European medicines legislation, as implemented in the national legislation of each country, nearly all antimicrobials for use in animals can only be used under prescription by a veterinarian. The treated animals must be in a real and practical sense be under the care of the prescribing veterinarian. This fact recognises the need for these products to be used with care if we are to maintain their efficacy and should help ensure that approved withdrawal periods are complied with. The only non-prescription antimicrobials are those used for "growth promotion" or, more correctly "digestive enhancement". No quinolone compounds are used for this purpose. The British Veterinary Poultry Association has recently adopted guidelines on the prescribing and use of antimicrobials in poultry (Appendix A). These guidelines in most regards merely document what has been normal practice for many years. It must be emphasised that the use of these products in poultry in North America and Europe is already much more restricted than that in other countries. The restrictions stem in part from the product approval process and in part from the system of veterinary prescribing. Most recently-licensed antimicrobials are approved for administration at a specific mg/kg liveweight as opposed to the previously common, but less accurate, approach of specifying a dosage per litre, or gallon of drinking water or a concentration in PPM of active in the drinking water. Variable water uptake according to age, species, environmental conditions and the disease we are treating makes it more difficult to ensure accurate dosing unless the dosage is calculated as mg.active kg/liveweight/day.

Pattern of use in our practice

Our practice deals with a mixture of broiler parent chickens, broilers and turkey growers. We have a computerised system of recording all medication and vaccines issued which allow us to both monitor individual farm usage and to look at trends over time. Figure 2 below shows the use of all soluble antimicrobials, as compared to the use of quinolones (both expressed as tonnes liveweight of bird medicated to take into account varying potency among products) and the pattern of vaccine use since this branch practice opened in January 1996. It will be noted that quinolone usage represented

![Diagram](image_url)

Figure 2 PHS2 Antimicrobial medication expressed as liveweight tonne/days

Month January 1996 to March 1997

- All antimicrobials  - Furoquinolone
only a small proportion of birds medicated (about 9% by weight). In fact only 1.85% by weight of the total poultry at risk in our practice are medicated with soluble antimicrobials at any given time, and of this only 0.165% is fluoroquinolone. For the purposes of this calculation the broiler parent chickens have been excluded since they rarely require medication. Total antibiotic medication varies markedly from month to month as a function of climate and disease challenge. There tends to be peak antimicrobial use in winter months. Vaccine usage is steadily increasing and it is hoped that this will result in reduced antimicrobial usage.

**Resistance patterns in isolates from our practice**

The great majority of bacterial isolates made from poultry flocks suffering clinical disease are *E. coli*. We routinely test all isolates for sensitivity to all antimicrobials in use by a disc-diffusion method. This is carried out by aseptically taking swabs from appropriate lesions (see Table 2 for species and affected organs) and seeding a “Diagnostic Sensitivity Test Agar” (Oxoid CM261), applying a range of disks containing candidate treatments (including enrofloxacin 5mg disks), and incubating for 18-24 hours at 37°C. At the same time the same sample is applied to blood agar and other appropriate selective media. If the culture turns out to be of mixed species or of inadequate density to read the sensitivity test the test is repeated with isolated colonies. Although inhibition zone sizes are not currently recorded, strains designated sensitive have zones over 15 mm in diameter, zones of about 10-15 mm are designated intermediate. In the first 9 months of using this system a number of *E. coli* cultures showed a mixed sensitivity profile with a few colonies growing within the area of the zone of inhibition. With “official” testing techniques, in which only a few isolated colonies are tested, these samples would probably be designated sensitive though we were recording them as resistant. To circumvent this problem we instituted a new category - “mixed sensitivity” with effect from September 1995. We have now extracted the sensitivity data for all *E. coli* isolates tested in 1996 and 1997. Table 2 shows the source of the isolates by species and organ. Figure 3 shows the number of isolates classified as fully sensitive, partially sensitive, of mixed sensitivity, and resistant to enrofloxacin. The percentage fully sensitive varies around 90% and shows no trend towards reduction, if anything the

<table>
<thead>
<tr>
<th>Species</th>
<th>1996</th>
<th>1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler Parent Chicken</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Broiler Chicken</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>Turkey Parent</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Turkey Grower</td>
<td>296</td>
<td>551</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organ</th>
<th>1996</th>
<th>1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>133</td>
<td>162</td>
</tr>
<tr>
<td>Liver</td>
<td>89</td>
<td>161</td>
</tr>
<tr>
<td>Pericardium</td>
<td>183</td>
<td>158</td>
</tr>
<tr>
<td>Air sac</td>
<td>114</td>
<td>127</td>
</tr>
<tr>
<td>Yolk</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Joint</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>peritoneum</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Abscess</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Oviduct</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S/c tissues</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tendon Sheath</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Trachea</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sinus</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Use of Quinolones in Food Animals and Potential Impact on Human Health
trend is towards improved sensitivity. The actual percentage of isolates classified as sensitive was 90.4%, while only 1.7% were recorded as resistant (though, as explained above, this includes a number of isolates which were, in fact, of mixed sensitivity in the period January-September 1995).

Figure 3 Post-mortem submissions - E.coli % sensitivity to Enrofloxacin by month

Discussion

Of the quinolone antimicrobials, the fluoroquinolones have particular advantages in the treatment of severe or multiple bacterial infections, particularly those of the respiratory system. Although these compounds are also highly effective for many Salmonella infections, certain strains (e.g. S. typhimurium DT104) are well recognised for their ability to become and remain resistant to a broad range of antimicrobials including quinolones. Their use where such infections are known or suspected to be present should be avoided. The spread of such strains through different animal species may have more to do with their inherent biology and colonising ability than actual usage of antimicrobials in these species. Concern has long been expressed about the possible reduction in sensitivity of Campylobacter sp. as a result of the use of fluoroquinolones in veterinary medicine (8). It would be somewhat surprising if their use in poultry had such an effect given the pattern of use of these products in breeding stock and very young commercial stock, since Campylobacter is not believed to be vertically transmitted and rarely appears in commercial broilers before 3 weeks of age. The prevalence of resistance in E. coli strains from our laboratory appears to be stable and considerably lower than that recently reported from Spain (9). While it is tempting to suggest that this reflects the more widespread use of both first and third generation quinolones in that country there are other possible explanations such as differing testing methodology, sources of isolates, etc. It must be accepted that selection of strains of reduced sensitivity or resistant strains is an inherent risk in any use of antimicrobials in animals or man. The maintenance of the efficacy of such products must be a key objective for all concerned. These hazards may be effectively control-
led in poultry medicine by a number of mechanisms:

1. Eradication of specific infections such as *Salmonella typhimurium*
2. Good clean-out and disinfection between crops to remove resistant strains
3. Effective vaccines and vaccination programmes to reduce the need for medication
4. Routine infection monitoring (e.g. *Salmonella* and *Campylobacter*) sensitivity testing of pathogens and tracking of results
5. Use of “normal intestinal flora” after medication to restore microbial balance.
6. Farm, hatchery and feed-mill “biosecurity” to prevent reintroduction of problem infections.

References
