2018

Technical Report on

critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis

World Health Organization

FIND
Because diagnosis matters
WHO collaborating centre for the evaluation of new diagnostic technologies

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Declaration and management of conflict of interest

All the contributors completed a WHO Declaration of Interest form. All stated declarations of interest were evaluated by members of the Steering Group for the existence of any possible financial conflict of interest which might warrant exclusion from membership of the Technical Expert Consultation Group or from the discussions as part of the consensus process. Intellectual conflict of interest was not considered for exclusion from membership of the Group, as broader expertise on DST

methods for MTBC was considered as criteria for the selection. In addition, the diversity and representation in the Groups was large enough to balance and overcome any potential intellectual conflict of interest. During the consensus development process and the meeting, any emergence of intellectual conflict of interest was monitored by the Chair and there was no perceived intellectual conflict of interest identified during the meeting.

The following interests were declared:

None declared
Christopher Coulter, Moses Joloba, Jacques Grosset, Lynette Berkeley, Sabira Tahseen, Armand Van Deun, Daniela Cirillo and Max Salfinger.

Declared, insignificant
Thomas Shinnick has declared that he was a former employee of the CDC until January 2016. As an employee, he had often represented CDC's positions on laboratory services needed for tuberculosis diagnosis, treatment and control.

Francis Drobniewski has declared that he was involved into the following activities: Grant to develop and implement a coordinated EU network of TB reference laboratories, ECDC, EUR 1.2 million; UK NIHR Health Technology Assessment and Innovate UK grants new TB diagnostic, systematic review and economic analysis modern TB diagnostics, £550,000; Unrestricted educational grant to develop and deliver training on clinical TB and MDR-TB management to medical doctors internationally, Otsuka, $75,000; EU FP7 grant PANNET; Research relating to MDR-TB diagnosis and management, EU FP7 grant, (approx. share of grant, Euro 1,000,000); Grant NIHR Imperial-PHE develop joint research between Imperial and PHE on DR, including TB, UK NIHR grants, £6,000; Grant UK Medical Research Council-evaluation of better physiological models for TB DR, UK MRC, £250,000; Activities of a consulting-training company providing training and supporting research in accord with WHO and international standards, total value £25,000.

Bernard Fourie has declared that he holds the position of Non-Executive Director of the South African National Bioproducts Institute (core business fractionation, manufacturing and supply of blood/plasma-related products), $10,000 per annum.

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Abbreviations

7H10	Middlebrook 7H10 medium
7H11	Middlebrook 7H11 medium
AMK	amikacin
BDQ	bedaquiline
CAP	capreomycin
CB	clinical breakpoint
CC	critical concentration
CDC	United States Centers for Disease Control and Prevention
CFZ	clofazimine
CI	exact binomial confidence interval
CLSI	Clinical & Laboratory Standards Institute
DCS	D-cycloserine
DLM	delamanid
DMSO	dimethyl sulfoxide
DST	drug-susceptibility testing
ECOFF	epidemiological cut-off value
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	United States Food & Drug Administration
FIND	Foundation for Innovative New Diagnostics
FQ	fluoroquinolone
gDST	genotypic drug-susceptibility testing
GFX	gatifloxacin
gNWT	genotypically non-wild type
GTB	Global TB Program
gWT	genotypically wild type
KAN	kanamycin
LFX	levofloxacin
LPA	line probe assay
LJ	Löwenstein-Jensen medium
LZD	linezolid
LOF	loss-of-function mutation (e.g. insertion, deletion or nonsense mutation)
MDR	multidrug-resistant

MFX	moxifloxacin
MGIT	BACTEC™ Mycobacterial Growth Indicator Tube™ 960
MIC	minimum inhibitory concentration
MTBC	*Mycobacterium tuberculosis* complex
OFX	ofloxacin
pDST	phenotypic drug-susceptibility testing
pNWT	phenotypically non-wild type
PMID	PubMed ID
pWT	phenotypically wild type
QC	quality control
QRDR	quinolone resistance-determining region
R	resistance/resistant
S	susceptible/susceptibility
SLI	second-line injectable (drug)
SOP	standard operating procedure
TB	tuberculosis
TEG	technical expert group
TRD	terizidone
v1	version 1
v2	version 2
WGS	whole genome sequencing
WHO	World Health Organization
XDR	extensively drug-resistant

Glossary of terms

Antimicrobial susceptibility test interpretive category – a classification based on an *in vitro* response of an organism to an antimicrobial agent. For mycobacteria, two different categories, "critical concentration" and "minimum inhibitory concentration", have been used to categorise the *in vitro* results. For strains of *Mycobacterium tuberculosis* complex, when tested against the lower concentration of some agents, the "critical concentration" category is applied. Testing of an additional higher concentration (a clinical breakpoint concentration) may also be recommended for some agents. However, there is no "intermediate" interpretive category, even when testing is performed both at the critical concentration and the clinical breakpoint concentration.

Critical concentration of an anti-tuberculous agent has been adopted and modified from international convention. The critical concentration is defined as the lowest concentration of an anti-TB agent *in vitro* that will inhibit the growth of 99% of phenotypically wild type strains of *M. tuberculosis* complex.

Clinical breakpoint – is the concentration or concentrations of an antimicrobial agent which defines an MIC above the critical concentration that separates strains that will likely respond to treatment from those which will likely not respond to treatment. This concentration is determined by correlation with available clinical outcome data, MIC distributions, genetic markers, and PK/PD data including drug dose. A dose increase can be used to overcome resistance observed at lower dosing, up until the maximum tolerated dose, and therefore a higher clinical breakpoint above which the particular drug is not recommended for use. The clinical breakpoint is used to guide individual clinical decisions in patient treatment. The clinical breakpoint is not applicable for drug resistance surveillance purposes.

Critical proportion – is the proportion of resistant organisms within a particular cultured isolate that is used to determine resistance to a particular drug. A 1% critical proportion is used to differentiate susceptible and resistant strains. Any culture that shows less than 1% growth on a medium containing a critical concentration of the agent being tested when compared with the growth on a control without the agent is considered to be susceptible; a culture that has 1% or more growth on the medium containing the critical concentration of the agent is considered to be resistant, and the patient whose sample is being tested may not respond to the agent. The critical concentration and proportion criteria are used for testing most first-line and second-line anti-TB agents.

Cross-resistance is resistance to multiple anti-tuberculosis agents caused by a single genetic change (or multiple changes, in case the given resistance mechanisms requires several genetic alterations), although in practice, such mutations may not be known.

Epidemiological cut-off value (ECOFF), phenotypically wild type (pWT) and non-wild type (pNWT) strains

- Typically, when MICs that are tested using a standardised method are aggregated for one species, a single Gaussian-shaped MIC distribution is formed, which corresponds to the **pWT** distribution for that species (i.e. the distribution for organisms that lack phenotypically detectable resistance mechanisms). Additional distributions with higher overall MICs are sometimes identified, even prior to the clinical use of the particular drug in question (or prior to the clinical use of another, related drug that shares the same resistance mechanism), that correspond to intrinsically or naturally resistant organisms. In this case, the distribution with the lowest MICs corresponds to the pWT distribution and the other distributions correspond to one or more **pNWT** distributions.
- The **ECOFF** corresponds to the upper end of the pWT distribution (i.e. it typically encompasses 99% of pWT strains).

- Excluding the scenario where it is difficult to distinguish pWT and pNWT strains because of methodological variation in MIC testing (i.e. where both distributions overlap), pWT strains are, by definition, genotypically WT (gWT). However, this does not mean that gWT strains are identical genotypically since they may harbour mutations in genes associated with resistance that do not change the MIC (e.g. the *gyrA* S95T mutation does not affect the MICs of fluoroquinolones).
- Conversely, organisms with MICs above the ECOFF are by definition pNWT. Again, excluding the possibility of methodological testing variation close to the ECOFF, there should be a genetic basis for this phenotype (i.e. the strains should be genotypically NWT (gNWT)). Yet in practice, these gNWT strains may appear to be gWT if:

  – The gene conferring the phenotype was not interrogated.

  – The gene was interrogated, but the genetic change conferring the phenotype was not detected, as it occurred at a frequency below the level of detection of the molecular test (i.e. heteroresistance).

  – The genetic change was detected but could not be interpreted because of an incomplete understanding of the genotype-phenotype relationship.

Indirect susceptibility test – a procedure based on inoculation of drug-containing media using organisms grown in culture.

Minimum inhibitory concentration (MIC) – the lowest concentration of an antimicrobial agent that prevents growth of more than 99% a microorganism in a solid medium or broth dilution susceptibility test.

Potency – All antimicrobial agents are assayed for standard units of activity or potency. The assay units may differ widely from the actual weight of the powder and often may differ between drug production lots. Thus, a laboratory must standardise its antimicrobial solutions based on assays of the antimicrobial powder lots that are being used.

The value for potency supplied by the manufacturer should include consideration for:

- Purity measures (usually by high-performance liquid chromatography assay)
- Water content (e.g. by Karl Fischer analysis or by weight loss on drying)
- Salt/counter-ion fraction (if the compound is supplied as a salt instead of free acid or base)

The potency may be expressed as a percentage, or in units of micrograms per milligrams (w/w).

Proportion method: The proportion method was originally proposed by Canetti and colleagues, and modified later; it is the most common method used for testing the susceptibility of *M. tuberculosis* complex isolates. In this method, the inoculum used is monitored by testing two dilutions of a culture suspension, and the growth (that is, the number of colonies) on a control medium without an anti-TB agent is compared with the growth (the number of colonies) present on a medium containing the critical concentration of the anti-TB agent being tested; the ratio of the number of colonies on the medium containing the anti-TB agent to the number of colonies on the medium without the anti-TB agent is calculated, and the proportion is expressed as a percentage. A 1% critical proportion is used differentiate the proportion of resistant organisms within a particular strain that is used to determine resistance to a particular drug.

Executive summary

The End TB Strategy calls for early diagnosis and prompt treatment of all persons of all ages with any form of drug-susceptible or drug-resistant TB. The effective management of multi-drug and extensively-drug resistant TB (M/XDR-TB) relies upon the rapid diagnosis and treatment of resistant infections. Culture-based phenotypic drug susceptibility testing (DST) methods are currently the gold standard for drug resistance detection although these methods are time-consuming; require sophisticated laboratory infrastructure, qualified staff and strict quality assurance mechanisms.

DST uses critical concentrations of anti-TB agents to determine the susceptibility or resistance of a culture of *Mycobacterium tuberculosis* complex. The critical concentration of an anti-TB agent has been adopted and modified from an international standard.[1] The critical concentration is defined as the lowest concentration of an anti-TB agent *in vitro* that will inhibit the growth of 99% of phenotypically wild type strains of *M. tuberculosis* complex.

Laboratory tests of the sensitivity of tubercle bacilli to anti-tuberculosis agents serve three main purposes. Firstly, they can be used as guidance in the choice of chemotherapy to be given to a patient. Secondly, they are of value in confirming that drug resistance has emerged when a patient failed to show a satisfactory response to treatment and thirdly can be used for the surveillance of emerging drug resistance.

WHO Global TB Programme commissioned FIND to perform a systematic review of available minimum inhibitory concentration (MIC) data for phenotypically wild type as well as phenotypically non-wild type strains, including associated sequencing data for relevant resistance genes. The medicines included in the review were the second-line injectable agents (kanamycin, amikacin and capreomycin), clofazimine and bedaquiline, cycloserine and terizidone, linezolid, delamanid, and the fluoroquinolones (ofloxacin, levofloxacin, gatifloxacin and moxifloxacin). The following media were considered: Löwenstein Jensen, Middlebrook 7H10/7H11 and BACTEC™ Mycobacterial Growth Indicator Tube™ 960.

In April 2017, WHO Global TB programme convened a Technical Expert Group to review the evidence for different critical concentrations and clinical breakpoints used for DST for the above-mentioned drug-media combinations. The revised or newly established breakpoints can be found in Table 1. A clinical breakpoint for the higher dose of moxifloxacin (800 mg/day) was established for the first time. Rifampicin and isoniazid critical concentrations were not evaluated as part of this review but should be re-evaluated as a priority. Finally, the Technical Expert Group highlighted the need for greater standardisation of DST protocols to minimise inter-laboratory differences.

Supplementary data. The supplementary document and data files for this report can be downloaded at https://www.finddx.org/publication/supplement-critical-concentrations-for-dst-for-tb-drugs.

1 Canetti, G. *et al.* Mycobacteria: laboratory methods for testing drug sensitivity and resistance. *Bull World Health Organ* 29, 565-78 (1963).

Table 1. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB.

Drug groups		Drug	LJ	7H10	7H11	MGIT [1]
A. Fluoroquinolones [2]		Levofloxacin (CC) [3]	2.0	1.0	–	1.0
		Moxifloxacin (CC) [3]	1.0	0.5	0.5	0.25
		Moxifloxacin (CB) [4]	–	2.0	–	1.0
		Gatifloxacin (CC) [3, 5]	0.5	–	–	0.25
B. Second-line injectable agents		Amikacin	30.0	2.0	–	1.0
		Capreomycin	40.0	4.0	–	2.5
		Kanamycin [6]	30.0	4.0	–	2.5
		(Streptomycin) [7]	4.0	2.0	2.0	1.0
C. Other second-line agents		Ethionamide [7]	40.0	5.0	10.0	5.0
		Prothionamide [7]	40.0	–	–	2.5
		Cycloserine / terizidone [8]	–	–	–	–
		Linezolid	–	1.0	1.0	1.0
		Clofazimine [9]	–	–	–	1.0
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide [7]	–	–	–	100.0
		Ethambutol [7]	2.0	5.0	7.5	5.0
	D2	Bedaquiline [9]	–	–	0.25	1.0
		Delamanid [9]	–	–	0.016	0.06
	D3 [10]	*p*-aminosalicylic acid [7]	–	–	–	–
		Imipenem-cilastatin [7]	–	–	–	–
		Meropenem [7]	–	–	–	–
		Amoxicillin-clavulanate [7]	–	–	–	–
		(Thioacetazone) [7]	–	–	–	–

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Unless otherwise stated, they are critical concentrations (CCs), as opposed to clinical breakpoints (CBs). Changes to the previous version of the table are highlighted in red.(11)

(1) MGIT is proposed as the reference method for performing DST for second-line anti-TB medicines.

(2) Testing of ofloxacin is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific fluoroquinolones used in treatment regimens. During this transition, testing of ofloxacin at the CCs (i.e. 4.0 mg/L on LJ, 2.0 mg/L on 7H10, 2.0 mg/L on 7H11 and 2.0 mg/L in MGIT) may be performed instead of testing at the CCs for levofloxacin, moxifloxacin and gatifloxacin, but not for the CBs for moxifloxacin.

(3) Levofloxacin, moxifloxacin and gatifloxacin interim CCs for LJ and gatifloxacin CC for MGIT established despite very limited data.

(4) CBs for 7H10 and MGIT apply to high-dose moxifloxacin (i.e. 800 mg daily).

(5) Gatifloxacin CC on 7H10 withdrawn due to limited evidence.

(6) Kanamycin CC on 7H11 withdrawn due to limited evidence.

(7) Drugs not reviewed as part of this report.

(8) Cycloserine CC on LJ withdrawn due to limited evidence.

(9) Interim CCs established.

(10) Routine DST is not recommended for Group D3 anti-TB medicines as these agents are only to be used when a MDR-TB treatment regimen with five effective medicines cannot otherwise be composed.

(11) World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014).

1. Introduction

1.0 Background

Tuberculosis (TB) causes 10 million cases and 1.3 million deaths annually and it is estimated that 4 million cases go undiagnosed by public health services each year.[2] Ending the global TB epidemic will be achievable over the next 20 years only if there is intensive action by all countries that have endorsed the End TB Strategy and its ambitious targets. It requires a paradigm shift from focused actions that gradually reduce the incidence of TB to enhanced, multisectoral actions that have been shown to drive down the epidemic at a rapid pace.

MDR-TB and XDR-TB are major global public health problems and threaten progress made in TB care and prevention in recent decades. Drug resistance in *Mycobacterium tuberculosis* complex (MTBC) is caused by selection of naturally occurring genomic mutants. There are two ways that people get drug-resistant TB (DR-TB). Firstly, acquired DR-TB occurs when TB treatment is suboptimal due to inadequate policies and failures of health systems and care provision, poor quality of TB drugs, poor prescription practices, patient non-adherence, or a combination of the above. Secondly, primary DR-TB results from the direct transmission of DR-TB from one person to another. Globally, 4.1% of new and 19.0% of previously treated TB cases were estimated to have had MDR-TB or rifampicin-resistant TB in 2016.[2] Each year MDR-TB or rifampicin-resistant TB leads to about 600,000 new cases and 240,000 deaths worldwide. Extensively drug-resistant TB (XDR-TB) has been reported by 123 countries. About 6.2% of patients with MDR-TB have XDR-TB globally. However, XDR-TB is more common among MDR-TB patients in some countries in Central Asia and Eastern Europe.

The End TB Strategy calls for early diagnosis and prompt treatment of all persons of all ages with any form of drug-susceptible or drug-resistant TB. This requires ensuring access to WHO-recommended rapid diagnostics and universal access to DST for all patients with signs and symptoms of TB and no longer only prioritised for persons at greater risk of multidrug-resistant TB (MDR-TB) and-or HIV-associated TB. WHO defines universal access to DST as rapid DST for at least rifampicin, and further DST for at least fluoroquinolones and second-line injectable agents among all TB patients with rifampicin resistance.[3]

The effective management of M/XDR-TB relies upon the rapid diagnosis and treatment of resistant infections. Culture-based phenotypic drug susceptibility testing (DST) methods are currently the gold standard for drug resistance detection, but these methods are time-consuming, require sophisticated laboratory infrastructure, qualified staff and strict quality control.

Traditionally, DST for MTBC has relied on the testing of a single, critical concentration (CC), which is used to differentiate resistant from susceptible strains of MTBC, and is specific for each anti-TB agent and test method. However, the definitions of CC for MTBC DST have evolved over time as have the definition of phenotypically wild type (pWT) vs. phenotypically non-wild type (pNWT) strains of MTBC.

Laboratory tests of the sensitivity of tubercle bacilli to anti-tuberculosis agents serve three main purposes. Firstly, they can be used as guidance in the choice of chemotherapy to be given to a patient. Secondly, they are of value in confirming that drug resistance has emerged when a patient failed to show a satisfactory response to treatment and thirdly can be used for the surveillance of emerging drug resistance.

1.1 Scope of the Technical Expert Consultation Meeting

The WHO GTB initiated and provided oversight to the process of evidence retrieval and analysis, was responsible for selection of members

2 Global tuberculosis report 2017. Geneva: World Health Organization; 2017 (WHO/HTM/TB/2017.23; http://www.who.int/tb/publications/global_report/en/, accessed 1 November 2017).

3 The End TB Strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2014 (http://www.who.int/tb/strategy/End_TB_Strategy.pdf, accessed 1 June 2017).

for the TEG and External Review Group, for management of declarations of interest, organising the preparatory TEG meetings via webinar, and, finally, conducting the face-to-face TEG meeting.

As a part of evidence retrieval and analysis, the WHO GTB commissioned the systematic review, which was performed by FIND in 2016-17. The aim of the review was to collect the available data on MICs of pWT and pNWT isolates, including associated sequencing data for relevant gene regions, for the following anti-TB drugs:

- SLIs (KAN, AMK and CAP)
- CFZ and BDQ
- DCS and TRD
- LZD
- DLM
- FQs (OFX, LFX, GFX and MFX)

The following media were considered:

- LJ
- 7H11
- 7H10
- MGIT

A series of the webinars took place between November 2016 and April 2017 as part of the preparatory process for the TEG meeting. During these webinars, a consensus was achieved for revised definitions for performing DST. These revised definitions are included in the glossary of the current report.

The objectives of the TEG were:

- To revise and update the CCs and CBs for performing culture-based DST for SLIs, FQs, DCS and TRD.
- To establish the CC for performing culture-based drug DST for the new anti-TB drugs (BDQ and DLM) and re-purposed agents (CFZ/TRD and LZD).

The TEG meeting was convened by the Global TB Programme, WHO on 24-26 April 2017 in Versoix, Switzerland. During that meeting, the group assessed the MIC and sequencing data for each drug-medium combination, with a particular focus on potential sources of bias, as outlined in the supplement. Depending on the quality and quantity of the data, CCs and/or CBs were either established, maintained or revised. Owing to the lack of data, the CCs were withdrawn in some cases. The decisions on the breakpoints for all anti-TB drugs in the review were based on consensus, which was defined as unanimous agreement among all TEG members. Consensus was achieved for all CCs and CBs both during the meeting and in consultations following the meeting. A CB for the higher dose of moxifloxacin (800 mg/day) was established for the first time.

The outcome of the TEG was an updated table of the CCs and CBs for the anti-TB agents recommended for the treatment of the rifampicin-resistant and/or MDR forms of TB, formatted in accordance with the recent guidance for the management of M/XDR-TB (Table 1).

Supplementary data. The supplementary document and data files for this report can be downloaded at https://www.finddx.org/publication/supplement-critical-concentrations-for-dst-for-tb-drugs.

1.2 Systematic review

1.2.1 Search methodology

A MEDLINE/PubMed search without date restrictions was conducted of all publications reporting quantitative DST results for the selected antibiotics. The search terms for each drug or group of drugs, which can be found in the supplement of this report, were intentionally broad since the titles or abstracts of papers do not necessarily mention MIC data. Moreover, MIC data were also solicited from the WHO Supranational Reference Laboratory Network and directly from key researchers, as identified through the literature search and laboratory network. Rifampicin and isoniazid were beyond the scope of the current review, but is envisaged to be assessed in phase 2 of the CC work and is planned for 2018.

Studies in the following languages were reviewed independently by two people, with the exception of studies published in Chinese or Russian, where each study was screened by a single person:

1. Chinese: Hairong Huang
2. English: Sophia Georghiou and Claudio Köser
3. French (a partial review of the literature):

Alexandra Aubry and Nicolas Veziris
4. German: Claudio Köser and Matthias Merker
5. Italian: Claudio Köser and Paolo Miotto
6. Russian: Alexei Korobitsyn or Vlad Nikolayevskyy
7. Spanish: Iñaki Comas and Sophia Georghiou

1.2.2 Inclusion criteria

Studies identified as containing any MIC data through the full-text screening were further reviewed in detail by Sophia Georghiou or Claudio Köser. Studies that met the following criteria were included in the review:

1. The MICs for at least one of the anti-TB compounds of interest (with at least three concentrations tested per drug) were determined using the proportion method with a critical proportion of 1%, using LJ, 7H10, 7H11 or MGIT.

2. The drug concentrations tested were clearly defined (i.e. to assess potential truncations of the MIC results).

3. The number of isolates tested at each concentration was given (i.e. to evaluate the shape of the MIC distributions and determine the mode of the distributions).

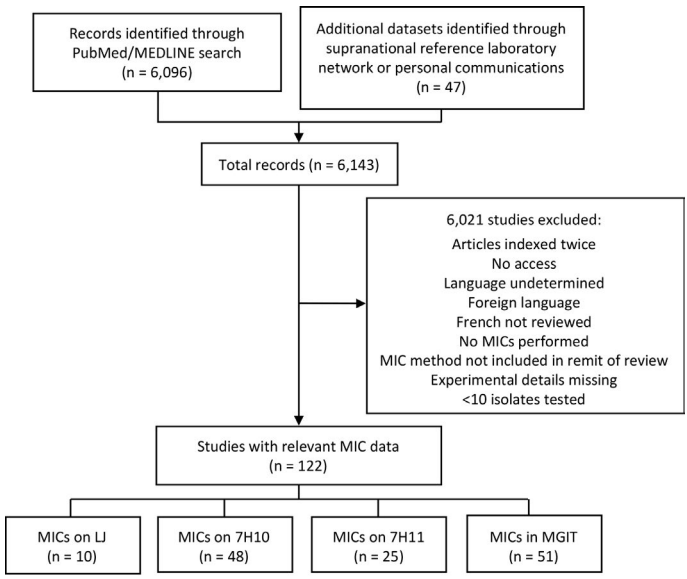
4. The MIC data were available for at least 10 isolates per drug.

For studies that reported only MIC ranges (i.e. did not meet the third criterion), raw study data were solicited directly from the corresponding authors and/or their co-authors. These studies were excluded if detailed MIC data could not be obtained. In exceptional circumstances, studies that did not meet all of these criteria were still included if they presented data that were particularly valuable, such as studies with sequencing data for new anti-TB drugs.

1.2.3 Studies identified through the systematic review

A total of 6,096 unique records were identified for possible inclusion, along with further 47 additional datasets from other sources. As shown in Figure 1, 122 of these studies were included in the review, which were stratified further by medium (NB: the sum of the studies for individual media does not correspond to 122 as some studies featured MICs for multiple media). The exclusion criteria in this diagram were not stratified in detail as a study may have been excluded for one drug but contained relevant data for another. This information can be found in the supplement, which contains PRISMA diagrams for each drug or group of drugs.

Figure 1. PRISMA diagram for overall search results and exclusion criteria



1.3 Data presentation

1.3.1 Format of this report

Each chapter in the report covers a single drug or group of drugs that share at least one resistance mechanism (e.g. the SLIs). The results in each chapter are grouped by different media (LJ, 7H10, 7H11 and MGIT). For each medium, data are organised into three sections: (1) MICs for pWT isolates, including laboratory control strains (e.g. H37Rv), (2) MICs for isolates with mutations in relevant resistance genes (i.e. MICs from *in vitro*, animal or clinical isolates as well as allelic exchange experiments, where available), (3) conclusion for CC and CB for each combination of drug and medium, including the rationale for revising and establishing breakpoints.

1.3.2 Format of MIC tables

This report contains abridged versions of the full Excel MIC data files, which are included in the supplement (an explanation of how to relate each table from this report with the raw data files can also be found in the supplement). Details for the information provided in each column of these files can be found below. However, only essential columns were included in this report. For example, the column with the 'total [number of] MICs' performed was included only if these numbers differed from the numbers of unique isolates tested (i.e. when isolates were tested repeatedly, as was the often the case for H37Rv).

The following points are relevant for the interpretation of the data:

- If a cell is empty, no information regarding the particular category were available (i.e. in the case of the 'genotypic results' column, blank cells are not equivalent to gWT (where sequencing or another genotypic method was carried out but no relevant genetic changes were found)).
- MICs from different studies cannot be compared unless the concentrations and ranges of concentrations tested are considered. Shaded cells therefore designate the concentrations tested for each group of isolates (NB: some studies tested a wide range of concentrations. The columns that correspond to the concentrations at the lower and upper end of these ranges were included but hidden in the Excel datasheets for simplicity. These columns can be displayed by highlighting the columns on either side of these hidden columns and using the 'unhide' command after a right-click). Table 2 provides an overview of how MIC data are displayed.

Table 2. Overview of MIC data presentation.

Studies	OFX MIC (mg/L)					
	0.5	1	2	4	6	8
study A			**15**	2	2	
study B	20		15			2

Shaded cells correspond to the concentrations tested in a particular study (e.g. concentrations of 1, 2 and 4 mg/L were tested for study A, whereas 1 was not tested in study B, which means that MICs of 2 mg/L in both studies are not equivalent). Truncated MIC values were highlighted in red. If red was used in a shaded cell, the MIC was either ≤ or ≥ the concentration in question. For example, the lowest MIC value for study B was ≤0.5 mg/L, whereas the highest MICs were ≥8 mg/L. If red was used in an unshaded cell, the MIC was > the last concentration tested (for study A, the highest MICs were >4 mg/L, as opposed to 6 mg/L). The mode of the putative pWT MIC distribution was indicated by highlighting the corresponding number of MICs in **bolded** text (e.g. 2 mg/L for study A). In the case of study B, the truncation of the MIC values meant that a mode could not be identified (e.g. it was possible that the MICs of all 20 isolates with MICs ≤0.5 mg/L were actually 0.5 mg/L, in which case 0.5 mg/L would be the mode of the MIC distribution).

The following information are provided in each data column.

'Studies' column:

- The names of the studies with notable limitations were highlighted in red (e.g. if the same laboratory participated in multiple studies that used the same medium or a method other than sequencing was used for genotypic DST). The corresponding limitations were detailed below the tables in the footnotes in this report and in the 'comment' column in red in the supplementary MIC file.

'Lab' column:
- The laboratories that participated in multiple studies using the same medium were highlighted in red.

'Unique isolates' & 'total MICs' columns:
- Red entries correspond to isolates that were tested multiple times.

'Genotypic results' column:
- In light of the recent endorsement of the Hain GenoType MTBDRs*l* v2 assay by WHO, the mutations that should be detected by this assay were noted.[4] Mutations in **bold** represent mutations targeted by specific mutant probes (i.e. they are specifically identified), whereas underlined mutations can only be inferred by the absence of binding of a wild type probe. For this purpose, it was assumed that any mutation in an area covered by a wild type probe would prevent binding of the probe, even if the mutation in question was not explicitly listed on the package insert of the assay. It should also be noted that the actual performance of this assay may differ from these simulations (e.g. the mutant probes may not always identify their respective mutations and the frequency of the mutation in the sample in question may affect the results).

'Comment' column:
- Additional remarks regarding the study in question were included in this column. Important limitations were highlighted in red.

4 Hain Lifescience. GenoType MTBDRs*l* VER 2.0. Instructions for use 06/2015. IFU-317A-02.

2. Second-line injectable agents

2.0 SLI MIC data stratification and current breakpoints

KAN, AMK and CAP MIC data were stratified by mutations in *rrs* (*MTB000019*), *eis* (*Rv2416c*), *tlyA* (*Rv1694*), *whiB7* (*Rv3197A*) and *rrl* (*MTB000020*) (details regarding these resistance mechanisms can be found in the supplement). The *M. tuberculosis* numbering system was used for the nucleotide changes in *rrs*, which were numbered relative to the start of the gene, and the data were stratified by the mutations A1401G, C1402T and G1484T. Only mutations that were within 50 nucleotides upstream of the *eis* start codon were reported. The inter-genic *whiB7* mutations were shown relative to the transcription start site of this gene (see supplement for more details). No assumptions were made about cross-resistance between KAN, AMK and CAP for this report, and therefore mutations in all resistance genes were analysed in relation to MIC data to all three drugs. Isolates with mutations in more than one resistance gene were excluded from the discussion, although these data are available in the supplementary MIC files.

Version 2 of the Hain GenoType MTBDR*sl* assay interrogates mutations in *rrs* and the *eis* promotor region, whereas version 1 only covers *rrs* (the mutations that are specifically targeted by mutation probes by this assay are heighted in **bold**, whereas mutations that merely inferred by lack of binding of a wild type probe are underlined both in the supplementary MIC files and in the tables of this report).[5]

Table 3 provides an overview of the current WHO and CLSI CCs for KAN, AMK and CAP.[6, 7]

Table 3. Overview of current SLI CCs.

	LJ		7H10		7H11		MGIT	
Drug	WHO	CLSI	WHO	CLSI	WHO	CLSI	WHO	CLSI
KAN	30.0	–	5.0		6.0		2.5	
AMK	30.0	–	4.0		–	–	1.0	
CAP	40.0	–	4.0	10.0	–	10.0	2.5	

Green CCs were set by both the WHO and CLSI; red CCs were set by WHO; blue CCs were set by CLSI. All concentrations are in mg/L.

2.A.1 KAN MIC data on LJ

2.A.1.1 KAN MICs for pWT isolates on LJ

Three studies from the same laboratory were identified that reported KAN MIC data for the pWT population on LJ (Table 4). Most MICs were truncated, which meant that little insight about the shape of the pWT MIC distribution could be gained.

5 Tagliani, E. *et al.* Diagnostic performance of the new version of GenoType MTBDR*sl* (V2.0) assay for detection of resistance to fluoroquinolones and second line injectable drugs: a multicenter study. *J Clin Microbiol* 53, 2961-9 (2015).
6 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).
7 World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1 (accessed 13.8.2015).

Table 4. KAN MICs for pWT isolates on LJ.

						KAN MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**7.5**	**15**	**30**	**60**	**120**	**128**
1) Jugheli 2009	1	clinical	76		gWT	41	13	9	3		10
2) Barletta 2014	1	clinical	7	MDR	gWT	2	**3**	2			
3) Vincent 2012	1	clinical	6	different levels of R	gWT	2	2	1	1		

The red line denotes the current WHO CC for KAN DST on LJ (30 mg/L). Notable limitation: All studies were performed in the same laboratory.

2.A.1.2 KAN MICs for mutated isolates on LJ

rrs mutants

Clinical isolates

Three studies from the same laboratory were identified that reported KAN MIC data for *rrs* mutants on LJ (Table 5). All 74 *rrs* A1401G mutants (100% (95% CI, 95-100%)) tested resistant at the current CC, whereas the four *rrs* C1402T mutants were susceptible (100% (95% CI, 40-100%)), which was not the case on 7H10 (Section 2.A.2.2).

Table 5. KAN MICs for clinical rrs mutants on LJ.

						KAN MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**7.5**	**15**	**30**	**60**	**120**	**128**
1) Jugheli 2009	1	clinical	65		*rrs* **A1401G**					1	64
2) Barletta 2014	1	clinical	4	MDR	*rrs* **A1401G**						4
3) Vincent 2012	1	clinical	5	different levels of R	*rrs* **A1401G**						5
1) Jugheli 2009	1	clinical	4		*rrs* C1402T		2	2			

The red line denotes the current WHO CC for KAN DST on LJ (30 mg/L). Notable limitation: All studies were performed in the same laboratory.

2.A.1.3 Conclusion for KAN CC for LJ

The identified KAN MIC data on LJ were limited and provided little insight into the shape of the pWT distribution, which precluded a re-assessment of the CC. Nevertheless, the current CC of **30 mg/L** was maintained given that the LJ proportion method is used widely and provides the only diagnostic option in some settings.

2.A.2 KAN MIC data on 7H10

2.A.2.1 KAN MICs for pWT isolates on 7H10

13 studies were identified that reported KAN MIC data for the pWT population on 7H10 (Table 6). Most distributions were not truncated and therefore provided a good understanding of the shape of the pWT MIC distributions. Some variation in testing was apparent, as some datasets had modes at 1 mg/L (e.g. Krüüner *et al.* & Engström *et al.* (Study 6)), for which a CC of 2 mg/L would be optimal. Others had pWT distributions that were slightly elevated (i.e. with a mode at 2 mg/L) and consequently supported a CC of 4 mg/L. In contrast to all other studies, the mode of Sowajassatakul *et al.* (Study 14) at 4 mg/L was high, with no MIC variation observed for the 28 pWT isolates that were tested in triplicate. Consequently, this study was excluded from all further analyses. Overall, these pWT MIC data supported lowering the current CC from 5 to 4 mg/L.

Table 6. KAN MICs for pWT isolates on 7H10.

							KAN MIC (mg/L)																				
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**0.62**	**1**	**1.25**	**2**	**2.5**	**4**	**5**	**8**	**10**	**16**	**20**	**32**	**40**	**64**	**80**	**128**	**256**	**512**	**1024**
4) Jureen 2010	2		1	4	H37Rv ATCC 27294						**3**		1															
	2	clinical	98	98	mostly pan-S				4		24		**61**		9													
5) Böttger	3		1	3	H37Rv ATCC 27294						**3**																	
	3		1	3	Erdman ATCC 35801						**3**																	
	3	clinical	84	84	pan-S or non-MDR			1	4		**59**		19		1													
6) Krüüner 2003 & Engström 2011	4	clinical	42	42		gWT		1	10		**25**		6															
	4		1	1	H37Rv ATCC 25618	gWT parent					**1**																	
	4		1	1	H37Ra ATCC 25177	gWT parent			**1**																			
	4		1	1	E55	gWT parent					**1**																	
	4		1	1	E3942	gWT parent							**1**															
	4		1	1	BTB 09-070	gWT parent							**1**															
	4		1	1	BTB 09-072	gWT parent					**1**																	
	4		1	1	BTB 09-116	gWT parent					**1**																	
7) Udou 2006	5	clinical	23	23	different levels of R						1		6		**9**		3				1				1			2
8) Pholwat 2011, 2012 & 2015	6		1	1	H37Rv ATCC 27294									**1**														
	6	clinical	35	35	different levels of R	gWT						**19**	13			3												
9) Hu 2013	7		1	1	H37Rv ATCC 25618						**1**																	
10) Reeves 2015	8		1	1	H37Rv	gWT							**1**															
	8		1	1	CDC1551	gWT							**1**															
	8		1	1	Beijing	gWT							**1**															
11) Zaunbrecher 2009	8		1	1	H37Rv	gWT							**1**															
	8	clinical	2	2		gWT										2												
12) Reeves 2013	8		1	1	H37Rv	gWT							**1**															
	8		1	1	CDC1551	gWT							**1**															
13) Pholwat 2016	9		1	1	H37Rv	gWT parent								**1**														
14) Sowajassatakul 2014	10		1	3	H37Rv ATCC 27294										**3**													
	10	clinical	27	81		gWT									**81**													
	10	clinical	3	6		gWT																		6				
15) Via 2010	11	clinical	89	89		gWT										86		2		1								
16) Maus 2005a	8	clinical	4	4		gWT												1				3						

The green line denotes the current WHO and CLSI CC for KAN DST on 7H10 (5 mg/L). Notable limitations: Studies 10-12 and 16 were performed in the same laboratory. Study 14 showed a high mode without any variability in the MIC distribution.

2.A.2.2 KAN MICs for mutated isolates on 7H10.

rrs mutants

Allelic exchange results

Reeves *et al.* (Study 10) conducted allelic exchange experiments in H37Rv, CDC1551 and Beijing strain backgrounds (Table 7). Compared to parental MICs of 2 mg/L, *rrs* A1401G conferred the largest MIC increase (>160 mg/L), followed by *rrs* G1484T (80-160 mg/L) and by *rrs* C1402T (10-15 mg/L).

Table 7. KAN MICs for rrs allelic exchange mutants on 7H10.

					KAN MIC (mg/L)												
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**1**	**2**	**3**	**4**	**5**	**10**	**15**	**20**	**40**	**80**	**100**	**160**	**256**
10) Reeves 2015	allelic exchange mutants	1	H37Rv	gWT		1											
		1		*rrs* **A1401G**													1
		1		*rrs* C1402T						1							
		1		*rrs* **G1484T**										1			
		1	CDC1551	gWT		1											
		1		*rrs* **A1401G**													1
		1		*rrs* C1402T							1						
		1		*rrs* **G1484T**												1	
		1	Beijing	gWT		1											
		1		*rrs* **A1401G**													1
		1		*rrs* C1402T							1						
		1		*rrs* **G1484T**												1	

The green line denotes the current WHO and CLSI CCs for KAN DST on 7H10 (5 mg/L).

In vitro and clinical isolates

All 199 *in vitro* or clinical *rrs* mutants tested resistant (100% (95% CI, 98-100%)), including the 11 *rrs* C1402T mutants (100% (95% CI, 72-100%)), which had the lowest KAN MICs (8-20 mg/L) (Table 8).

Table 8. KAN MICs for in vitro and clinical rrs mutants on 7H10.

						KAN MIC (mg/L)															
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**4**	**5**	**8**	**10**	**12**	**16**	**20**	**32**	**40**	**64**	**80**	**96**	**128**	**160**	**256**	**320**
6) Krüüner 2003 & Engström 2011	4	clinical	54		*rrs* **A1401G**													1	53		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	4		*rrs* **A1401G**														4		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	1		*rrs* **A1401G**														1		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2		*rrs* **A1401G**														2		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	1		*rrs* **A1401G**														1		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	5		*rrs* **A1401G**													2	3		
8) Pholwat 2011, 2012 & 2015	6	clinical	1	different levels of R	*rrs* **A1401G**					1											
9) Hu 2013	7	clinical	40		*rrs* **A1401G**						1		2		9			20		6	2
15) Via 2010	11	clinical	16		*rrs* **A1401G**												16				
16) Maus 2005a	8	*in vitro* mutants	15	H37Rv or CDC1551	*rrs* **A1401G**												15				
16) Maus 2005a	8	clinical	11		*rrs* **A1401G**												11				
17) Maus 2005b	8	clinical	13		*rrs* **A1401G**												13				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	4		*rrs* C1402T			4													
9) Hu 2013	7	clinical	2		*rrs* C1402T						2										
16) Maus 2005a	8	*in vitro* mutants	5		*rrs* C1402T				3			2									
8) Pholwat 2011, 2012 & 2015	6	clinical	1	different levels of R	*rrs* **G1484T**					1											
16) Maus 2005a	8	*in vitro* mutants	23		*rrs* **G1484T**												23				
16) Maus 2005a	8	clinical	1		*rrs* **G1484T**												1				

The green line denotes the current WHO and CLSI CCs for KAN DST on 7H10 (5 mg/L). Notable limitation: Studies 16 and 17 were conducted in the same laboratory.

eis mutants

Allelic exchange results

Zaunbrecher *et al.* (Study 11) were the first to study *eis* mutations (Table 9). The introduction of the *eis* C-14T promoter mutation into an H37Rv background increased the KAN MIC from 2 mg/L to 20-25 mg/L. Conversely, the restoration of the *eis* wild type sequence in an *eis* C-14T *in vitro* mutant lowered the MIC from 25 back to 2 mg/L. Zaunbrecher *et al.* further investigated this KAN resistance mechanism by complementing an unmarked *eis* deletion mutant of H37Rv with five different promoter mutations. For *eis* G-10A, C-12T, C-14T, and G-37T this resulted in KAN MICs of 10-20 mg/L. The MIC of the A-13G mutant was 5 mg/L, which is equal to the current WHO CC for this medium, but above the lowered CC of 4 mg/L.

Pholwat *et al.* (Study 13) also conducted allelic exchange experiments in H37Rv, which confirmed that the aforementioned *eis* G-10A, C-12T, and C-14T mutations resulted in a KAN MIC increase above the current CC of 5 mg/L on 7H10 (i.e. from 2.5 mg/L to 10-40 mg/L). Notably, the C-14T mutation resulted in the largest KAN MIC increase (i.e. to 40 mg/L), as seen by Zaunbrecher *et al.* In addition, Pholwat *et al.* investigated two *eis* promoter mutations that were not evaluated by Zaunbrecher *et al.* Of these, the *eis* C-14G mutation did not change the KAN MIC compared to the wild type parent (2.5 mg/L), whereas the MIC of the *eis* C-15G mutation increased by one doubling dilution, which was within the normal variation of MIC testing. Pholwat *et al.* therefore concluded that these two mutations did not confer KAN resistance.

Table 9. KAN MICs for eis allelic exchange mutants on 7H10.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	KAN MIC (mg/L) 1	1.25	2	2.5	4	5	10	15	20	25	30	40
11) Zaunbrecher 2009	allelic exchange mutants & complemented strains	1	H37Rv	gWT			**1**									
		2		*eis* **C-14T** introduced by alleleic exchange									1	1		
		1	H37Rv	*eis* **C-14T** *in vitro* mutant										1		
		2		gWT (*eis* mutation removed by allelic exchange)			2									
		1	H37Rv Δ*eis*	complemented with WT *eis*			1									
		1		complemented with *eis* G-10A							1					
		1		complemented with *eis* C-12T							1					
		1		complemented with *eis* A-13G						1						
		1		complemented with *eis* **C-14T**									1			
		1		complemented with *eis* G-37T									1			
13) Pholwat 2016	allelic exchange mutants	1	H37Rv	gWT parent				**1**								
		1		gWT recombinant				1								
		3		*eis* G-10A introduced by alleleic exchange									3			
		3		*eis* C-12T introduced by alleleic exchange							3					
		3		*eis* C-14G introduced by alleleic exchange				3								
		3		*eis* **C-14T** introduced by alleleic exchange												3
		2		*eis* C-15G introduced by alleleic exchange						2						

The green line denotes the current WHO and CLSI CC for KAN DST on 7H10 (5 mg/L).

In vitro and clinical isolates

Four studies reported MIC data for 83 *in vitro* or clinical *eis* mutants without known mutations in other resistance genes (Table 10). 36 of these isolates (43% (95%CI, 33-55%)) were susceptible at the current CC. This was mainly due to *eis* G-10A mutants from Krüüner *et al.* & Engström *et al.* (Study 6). Notably, most of these isolates would have tested resistant at 2 mg/L, the optimal CC for this particular dataset (Section 2.A.2.1). However, even at 2 mg/L some *eis* G-10A mutants would have been misclassified as susceptible given that the lower end of the MIC distribution of this mutation was also 2 mg/L. By contrast, the variation in testing between datasets had less of an impact on the *eis* C-14T mutation (i.e. only 1 of the 25 mutants (4% (95% CI, 0-20%)) tested susceptible), as this mutation conferred larger MIC increases, which was in agreement with the allelic exchange data.

Table 10. KAN MICs for in vitro and clinical eis mutants on 7H10.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	KAN MIC (mg/L) 1	2	4	5	8	10	15	16	20	25	30	32	40	64	80
6) Krüüner 2003 & Engström 2011	clinical	35		*eis* G-10A		1	27		6			1							
9) Hu 2013	clinical	9		*eis* G-10A		1	1									7			
11) Zaunbrecher 2009	clinical	3		*eis* G-10A						2									1
6) Krüüner 2003 & Engström 2011	clinical	2		*eis* C-12T			2												
11) Zaunbrecher 2009	clinical	2		*eis* C-12T				1		1									
11) Zaunbrecher 2009	clinical	1		*eis* A-13G				1											
6) Krüüner 2003 & Engström 2011	clinical	10		*eis* **C-14T**			1		2			7							
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	1		*eis* **C-14T**					1										
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	3		*eis* **C-14T**								3							
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	1		*eis* **C-14T**								1							
9) Hu 2013	clinical	6		*eis* **C-14T**					1							5			
11) Zaunbrecher 2009	*in vitro* mutants	1	H37Rv	*eis* **C-14T**										1					
11) Zaunbrecher 2009	clinical	2		*eis* **C-14T**													2		
13) Pholwat 2016	clinical	1		*eis* **C-14T**													1		
6) Krüüner 2003 & Engström 2011	clinical	1		*eis* C-15G			1												
6) Krüüner 2003 & Engström 2011	clinical	2		*eis* G-37T					1			1							
11) Zaunbrecher 2009	clinical	3		*eis* G-37T						1			2						

The green line denotes the current WHO and CLSI CCs for KAN DST on 7H10 (5 mg/L).

whiB7 mutants

Allelic exchange results

Reeves *et al.* (Study 12) conducted allelic exchange experiments to restore the wild type *whiB7* sequence in three *in vitro* mutants that had KAN MICs of 10-20 mg/L (Table 11). This resulted in KAN MICs in the susceptible range (2-4 mg/L).

Table 11. KAN MICs for whiB7 allelic exchange, in vitro and clinical mutants on 7H10.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	KAN MIC (mg/L) 1	2	3	4	5	10	15	20
12) Reeves 2013	allelic exchange mutants	1	H37Rv	*whiB7* C+134 *in vitro* mutant								1
		1		gWT (*whiB7* mutation removed)				1				
		1	H37Rv	*whiB7* A+238G *in vitro* mutant						1		
		1		gWT (*whiB7* mutation removed)		1						
		1	CDC1551	*whiB7* ΔC+134 *in vitro* mutant								1
		1		gWT (*whiB7* mutation removed)		1						
	in vitro mutants	1	H37Rv	gWT		**1**						
		2		*whiB7* mutants						1		1
		1	CDC1551	gWT		**1**						
		8		*whiB7* mutants								8
	clinical	1		*whiB7* C+134								1

The green line denotes the current WHO and CLSI CCs for KAN DST on 7H10 (5 mg/L).

In vitro and clinical isolates

10 additional *whiB7 in vitro* mutants obtained during the selection experiments had MICs of 10-20 mg/L, compared with 2 mg/L for the two parental strains (Table 11). The sole clinical mutant had an MIC of 20 mg/L.

tlyA mutants

In vitro and clinical isolates

38 *in vitro* and clinical isolates with *tlyA* mutations were reported by three studies from two laboratories (Table 12). Of those, 34 (89% (95% CI, 75-97%)) were susceptible at the current CC for KAN.

Table 12. KAN MICs for in vitro and clinical tlyA mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Genotypic results	KAN MIC (mg/L) 0.25	0.5	1	2	4	5	8	10	16	20	32	40	64	80
6) Krüüner 2003 & Engström 2011	4	clinical	4	*tlyA* mutants		1	2	1										
17) Maus 2005b	8	clinical	4	*tlyA* mutants						2				2				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	8	*tlyA* mutants				4	4									
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	5	*tlyA* mutants			2	3										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants				2										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	3	*tlyA* mutants					3									
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants				2										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants				2										
16) Maus 2005a	8	*in vitro* mutants	8	*tlyA* mutants						6		1				1		

The green line denotes the current WHO and CLSI CC for KAN DST on 7H10 (5 mg/L). Notable limitation: Studies 16 and 17 were conducted in the same laboratory.

2.A.2.3 Conclusion for KAN CC for 7H10

Few studies included 5 mg/L, the current KAN CC for 7H10, in their dilution series. Instead, **4 mg/L** was adopted as the new CC to maximise the detection of *eis* mutants as well as yet unknown resistance mechanisms. This change should not affect the detection of *rrs* mutants, which reliably tested resistant even at 5 mg/L. Lowering the CC should also not result in the misclassification of *tlyA* mutants as resistant.

Because of the variation between laboratories and/or different datasets and the fact that the MIC distributions for pWT isolates and some *eis* mutants overlapped (even when tested in the same laboratory), these *eis* mutants cannot be detected reliably using pDST. The implications of this finding for the interpretation of gDST results will be addressed in a later report. Based on the limited MIC data for *whiB7* mutants and the fact that these mutants confer resistance through the over-expression of eis, it is possible that the ability of pDST to detect this class of mutants is also compromised.

2.A.3 KAN MIC data on 7H11

2.A.3.1 KAN MICs for pWT isolates on 7H11

Only one study was identified with KAN MIC data for 7H11 (Table 13).

Table 13. KAN MICs for pWT isolates on 7H11.

				KAN MIC (mg/L)							
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**0.75**	**1.5**	**3**	**6**	**12**	**24**	**48**	**96**
18) Fattorini 1999		1	H37Rv ATCC 27294		**1**						
	clinical	46	R to at least 2 first-line drugs		10	**18**	9				9

The green line denotes the current WHO and CLSI CC for KAN DST on 7H11 (6 mg/L)

2.A.3.2 KAN MICs for mutated isolates on 7H11

No studies presenting MIC data for mutants were identified.

2.A.3.3 Conclusion for KAN CC for 7H11

Given that KAN MIC data were only identified for a single study (which precluded an analysis of the inter-laboratory reproducibility) and that CCs for KAN for other media are supported by more evidence, the current CC of 6 mg/L was withdrawn.

2.A.4 KAN MIC data in MGIT

2.A.4.1 KAN MICs for pWT isolates in MGIT

10 studies were identified that reported KAN MIC data for the pWT population in MGIT (Table 14). Most studies used non-standard MIC dilution series as the current CC is 2.5 mg/L. Several studies were severely truncated at the lower end, including Rodrigues *et al.* (Study 26), which has been cited by the current CLSI document to support the current CC of 2.5 mg/L.[8] Nevertheless, variation in testing was identifiable given that some studies had modes at 0.62 mg/L (e.g. Study 21) whereas others had modes that were one dilution higher, at 1.25 mg/L (e.g. Study 19). The current CC appeared to balance this variation appropriately, although more genotypic data for isolates with MICs just above this concentration is desirable to investigate the true upper end of the pWT distribution (e.g. in Study 22).

8 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Table 14. KAN MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	KAN MIC (mg/L) 0.31	0.5	0.62	1	1.25	2	2.5	3	4	5	6	7.5	8	10	12	12.5	16	20	24	32	40	50	60	64	80	96	128
19) Tessema 2017	13	clinical	40	40		gWT					**22**		15			2						1											
20) Heyckendorf 2017	13		1	1	H37Rv ATCC 27294						**1**																						
	13	clinical	9	9	MDR or XDR	gWT			2		**7**																						
21) Stepanshina 2009	14		1	1	H37Rv				**1**																						
	14	clinical	27	27	different levels of R	gWT	9		**15**				2			1																
22) Zheng 2016	7		1	1	H37Rv ATCC 25618					**1**																						
	7	clinical	207	207	MDR			23		**46**		28			13				50			5			16				8			18
23) Gikalo 2012 & Zimenkov 2013	15		1	1	H37Rv ATCC 25618								**1**																			
	15	clinical	44	44		gWT			11		**28**		3			1															1	
24) Kambli 2016a & 2016b	16		1	1	H37Rv ATCC 27294				1																						
	16	clinical	31	31		gWT			30											1												
25) Bastian 2001	1		3	3	KAN-S ATCC strains					1			2																			
	1		1	1	KAN-R ATCC 35827 strain																								1			
	1	clinical	6	6						2			3																1			
	1		1	1																					1							
26) Rodrigues 2008	16		1	1	H37Rv ATCC 27294						1																					
	16	clinical	10	10	pan-S						10																					
	16		20	20	different levels of R						13		4			2	1															
27) Lin	17		1	45	H37Rv ATCC 27294						25		20																			
	17	clinical	114	114							58		11	1	3	1	1	2		2	35											
28) Sharma 2011	18	clinical	36	36	different levels of R								25			1			10													

The green line denotes the current WHO and CLSI CC for KAN DST in MGIT (2.5 mg/L). Notable limitations: Studies 19 and 20, and Studies 24 and 26 were performed in the same laboratory, respectively. The genotypic results for Study 23 were based on a combination of sequencing and a microarray, whereas Study 24 relied on a combination of sequencing and the MTBDRs*l* v1.

2.A.4.2 KAN MICs for mutated isolates in MGIT

rrs mutants

Clinical isolates

Five studies conducted in four laboratories interrogated 105 clinical isolates harbouring the *rrs* A1401G mutation (Table 15). 100% (95% 97-100%) tested resistant at the current CC with MICs of >20 mg/L.

Table 15. KAN MICs for clinical rrs mutants in MGIT.

					KAN MIC (mg/L)											
Studies	Lab	Isolate origin	Unique isolates	Genotypic results	1.25	2.5	5	10	12.5	20	24	25	30	40	80	96
19) Tessema 2017	13	clinical	3	*rrs* **A1401G**									3			
20) Heyckendorf 2017	13	clinical	4	*rrs* **A1401G**									4			
21) Stepanshina 2009	14	clinical	18	*rrs* **A1401G**												
23) Gikalo 2012 & Zimenkov 2013	15	clinical	32	*rrs* **A1401G**												32
24) Kambli 2016a & 2016b	16	clinical	48	*rrs* **A1401G**							48					

The green line denotes the current WHO and CLSI CCs for KAN DST in MGIT (2.5 mg/L). Notable limitations: Studies 19 and 20 were performed in the same laboratory. The genotypic results for Study 23 were based on a combination of sequencing and a microarray, whereas Study 24 relied on a combination of sequencing and the MTBDRs*l* v1.

eis mutants

Clinical isolates

Four studies from three laboratories reported MIC data for 42 *eis* mutants that would be interpreted as resistant by MTBDRs*l* v2 assay (Table 16). Of these, seven (17% (95% CI, 7-31%)) were susceptible at the current CC. Notably, two of the 'susceptible' isolates (one *eis* G-10A and C-10C mutant) from Kambli *et al.* (Study 24) were tested in a laboratory with a relatively low pWT MIC distribution (Section 2.A.4.1) and still had clearly elevated MICs.

Table 16. KAN MICs for clinical eis mutants in MGIT.

					KAN MIC (mg/L)											
Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.62	1.25	2.5	5	10	12.5	20	25	40	80	96	
20) Heyckendorf 2017	13	clinical	3	*eis* G-10A				2		1						
23) Gikalo 2012 & Zimenkov 2013	15	clinical	10	*eis* G-10A				1	8						1	
24) Kambli 2016a & 2016b	16	clinical	3	*eis* G-10A			1	1	1							
24) Kambli 2016a & 2016b	16	clinical	3	*eis* G-10C			1	2								
19) Tessema 2017	13	clinical	1	*eis* C-12T				1								
23) Gikalo 2012 & Zimenkov 2013	15	clinical	2	*eis* C-12T		1		1								
24) Kambli 2016a & 2016b	16	clinical	2	*eis* C-12T				2								
19) Tessema 2017	13	clinical	1	*eis* **C-14T**		1										
23) Gikalo 2012 & Zimenkov 2013	15	clinical	7	*eis* **C-14T**	1						5		1			
24) Kambli 2016a & 2016b	16	clinical	3	*eis* **C-14T**	1				2							
19) Tessema 2017	13	clinical	1	*eis* G-37T						1						
20) Heyckendorf 2017	13	clinical	1	*eis* G-37T			1									
23) Gikalo 2012 & Zimenkov 2013	15	clinical	5	*eis* G-37T				1	3		1					

The green line denotes the current WHO and CLSI CCs for KAN DST in MGIT (2.5 mg/L). Notable limitations: Studies 19 and 20 were performed in the same laboratory. The genotypic results for Study 23 were based on a combination of sequencing and a microarray, whereas Study 24 relied on a combination of sequencing and the MTBDRs*l* v1.

whiB7 mutant

Clinical isolate

One clinical mutant that tested KAN susceptible in MGIT (with an MIC equal to the CC of 2.5 mg/L) harboured a *whiB7* A+238G mutation (Table 17). Reeves *et al.* (Study 12) had previously demonstrated that the removal of this mutation in an *in vitro* mutant restored KAN susceptibility on 7H10 (Section 2.A.2.2). Consequently, the MIC of this mutant might be close to the CC and might become resistant upon retesting as a result of the normal variation in pDST.

Table 17. KAN MICs for clinical whiB7 mutant in MGIT.

				KAN MIC (mg/L)				
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**1.25**	**2.5**	**5**	**12.5**	**25**
20) Heyckendorf 2017	clinical	1	*whiB7* A+238G		1			

The green line denotes the current WHO and CLSI CCs for KAN DST in MGIT (2.5 mg/L).

2.A.4.3 Conclusion for KAN CC in MGIT

The current CC of **2.5 mg/L** is likely appropriate to balance the variation in MIC testing and was consequently reaffirmed. As was the case for 7H10, the detection of *eis* mutants, but not *rrs* mutants, was likely affected by variation in MIC testing.

2.A.5 References for KAN MIC studies

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2.B.1 AMK MIC data on LJ

2.B.1.1 AMK MICs for pWT isolates on LJ

Four studies from two laboratories were identified that reported AMK MIC data for the pWT population on LJ (Table 18). These data provided little insight into the shape of the pWT distribution due to truncations at the lower end of the distributions.

Table 18. AMK MICs for pWT isolates on LJ.

						AMK MIC (mg/L)										
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**2**	**7.5**	**8**	**10**	**15**	**20**	**30**	**32**	**40**	**60**	**120**	**128**
1) Fabry 1995	1	clinical	20			20											
2) Jugheli 2009	2	clinical	76		gWT		55			11		5		1			4
3) Barletta 2014	2	clinical	7	MDR	gWT				3		4						
4) Vincent 2012	2	clinical	6	different levels of R	gWT				6								

The red line denotes the current WHO CC for AMK DST on LJ (30 mg/L). Notable limitation: Studies 2, 3 and 4 were performed in the same laboratory.

2.B.1.2 AMK MICs for mutated isolates on LJ

rrs mutants

Clinical isolates

Based on data from a single laboratory (Table 19), most *rrs* A1401G mutants (99% (95% CI, 93-100%)) were resistant at the current CC, whereas all four *rrs* C1402T mutants were susceptible (100% (95% CI, 40-100%)).

Table 19. AMK MICs for clinical rrs mutants on LJ.

						AMK MIC (mg/L)											
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**7.5**	**10**	**15**	**20**	**30**	**40**	**60**	**80**	**120**	**128**	**160**	**256**
2) Jugheli 2009	2	clinical	65		*rrs* **A1401G**										65		
3) Barletta 2014	2	clinical	4	MDR	*rrs* **A1401G**												4
4) Vincent 2012	2	clinical	5	different levels of R	*rrs* **A1401G**				1								4
2) Jugheli 2009	2	clinical	4		*rrs* C1402T	1		3									

The red line denotes the current WHO CC for AMK DST on LJ (30 mg/L). Notable limitation: All studies were performed in the same laboratory.

2.B.1.3 Conclusion for AMK CC on LJ

The identified AMK MIC data for LJ were limited and provided little insight into the shape of the pWT distribution, which precluded a re-assessment of the CC of **30 mg/L**. Nevertheless, this CC was maintained as LJ represents the only medium available for pDST in many settings.

2.B.2 AMK MIC data on 7H10

2.B.2.1 AMK MICs for pWT isolates on 7H10

14 studies were identified that reported AMK MIC data for the pWT population on 7H10 (Table 20). Where the MIC distributions were not truncated, the modes varied between 0.5 to 2 mg/L. The distribution of Sowajassatakul *et al.* (Study 16) was particularly high, as also seen for KAN on 7H10 (Section 2.A.2.1). This study was therefore excluded from additional analyses. The remaining studies, apart from van Ingen *et al.* (Study 14), suggested that the CC should be lowered from 4 to 2 mg/L.

2.B.2.2 AMK MICs for mutated isolates on 7H10

rrs mutants

Allelic exchange results

Reeves *et al.* (Study 11) conducted allelic exchange experiments in H37Rv, CDC1551 and Beijing strain backgrounds (Table 21). The MICs of the three parent strains were 0.5-1 mg/L, compared to 2-4 mg/L for *rrs* C1402T, 100 mg/L for *rrs* G1484T and >128 mg/L for *rrs* A1401G. Lowering the CC from 4 to 2 mg/L meant that the interpretation of *rrs* C1402T, which is currently not regarded as a resistance mutation for AMK, should be reviewed.

Table 21. AMK MICs for rrs allelic exchange mutants on 7H10.

					AMK MIC (mg/L)											
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**100**	**128**	**160**
11) Reeves 2015	allelic exchange mutants	1	H37Rv	gWT		**1**										
		1		*rrs* **A1401G**												1
		1		*rrs* C1402T				1								
		1		*rrs* **G1484T**										1		
		1	CDC1551	gWT			**1**									
		1		*rrs* **A1401G**												1
		1		*rrs* C1402T					1							
		1		*rrs* **G1484T**										1		
		1	Beijing	gWT			**1**									
		1		*rrs* **A1401G**												1
		1		*rrs* C1402T					1							
		1		*rrs* **G1484T**										1		

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L).

In vitro and clinical isolates

Seven studies from six laboratories presented MIC data for *in vitro* or clinical *rrs* mutants (Table 22). All 193 *rrs* A1401G or G1484T mutants (100% (95% CI, 98-100%)) were resistant at the current CC. The three studies that tested wide concentration ranges for the *rrs* C1402T mutations showed more varied results. The two mutants from Hu *et al.* (Study 13) and three mutants from van Ingen *et al.* (Study 14) had significantly higher MICs (32 mg/L and >20 mg/L, respectively) than the five MICs from Krüüner *et al.* & Engström *et al.* (Study 7), which ranged from 1-4 mg/L (with a mode at 2 mg/L). It is therefore possible that an unidentified mutation was responsible for the high MIC in Study 13. Similarly, it is possible that the isolates from van Ingen *et al.* actually harboured *rrs* A1401G mutations, given that the *rrs* C1402T was only inferred using the Hain Genotype MTBDR*sl* v1 assay (the authors noted that the hybridisation of the A1401G probe was generally weak in their study). By contrast, the MICs from Study 7 confirmed the allelic exchange results for *rrs* C1402T given that the corresponding distribution (1-4 mg/L, with a mode at 2 mg/L) was clearly elevated compared to the pWT MIC distribution in this dataset, which ranged from 0.12-1 mg/L (Section 2.B.2.1).

Table 20. AMK MICs for pWT isolates on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	AMK MIC (mg/L) 0.12	0.25	0.31	0.5	0.62	1	1.25	2	2.5	4	5	8	10	16	32	64	80	128	512	1024
5) Juréen 2010	3		1	4	H37Rv ATCC 27294					**4**																
	3	clinical	98	98	mostly pan-S			2		40		**56**														
6) Böttger	4		1	3	H37Rv ATCC 27294					**3**																
	4		1	3	Erdman ATCC 35801					**3**																
	4	clinical	87	87	pan-S or non-MDR		1	2		**61**		22		1												
7) Krüüner 2003 & Engström 2011	5	clinical	42	42		gWT	1	9		**21**		11														
	5		1	1	H37Rv ATCC 25618	gWT parent						**1**														
	5		1	1	H37Ra ATCC 25177	gWT parent				**1**																
	5		1	1	E55	gWT parent		**1**																		
	5		1	1	E3942	gWT parent				**1**																
	5		1	1	BTB 09-070	gWT parent						**1**														
	5		1	1	BTB 09-072	gWT parent						**1**														
	5		1	1	BTB 09-116	gWT parent				**1**																
8) Pholwat 2011, 2012 & 2015	6		1	1	H37Rv ATCC 27294								**1**													
	6	clinical	16	16	different levels of R	gWT					3		**13**													
	6		19	19	different levels of R	gWT		1		**18**																
9) Udou 2006	7	clinical	23	23	different levels of R					1		9		**10**										1		2
10) de Steenwinkel 2012	8		1	2	H37Rv ATCC 27294									**2**												
	8	clinical	10	20						1		**10**		9												
11) Reeves 2015	9		1	1	H37Rv	gWT				**1**																
	9		1	1	CDC1551	gWT						**1**														
	9		1	1	Beijing	gWT						**1**														
12) Zaunbrecher 2009	9		1	1	H37Rv	gWT parent				**1**																
13) Hu 2013	10		1	1	H37Rv ATCC 25618					1																
14) van Ingen 2010	11		1	1	H37Rv ATCC 27294							1														
	11	clinical	20	20	MDR	gWT						3		**16**			1									
15) Pholwat 2016	12		1	1	H37Rv	gWT parent						**1**														
16) Sowajassatakul 2014	13		1	3	H37Rv ATCC 27294									3												
	13	clinical	27	81		gWT								50		31										
	13		3	6		gWT																	6			
17) Via 2010	14	clinical	90	90		gWT										89					1					
18) Maus 2005a & Reeves 2013	9	clinical	4	4		gWT										3		1								

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L). Notable limitations: Studies 11, 12 and 18 were performed in the same laboratory. The genotypic results for Study 14 were based on the MTBDRs/ v1. The MIC distribution for Study 16 was unusually high.

Table 22. AMK MICs for in vitro and clinical rrs mutants on 7H10.

						AMK MIC (mg/L)																	
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.5**	**1**	**2**	**2.5**	**4**	**5**	**8**	**10**	**15**	**16**	**20**	**30**	**32**	**64**	**80**	**128**	**160**	**256**	**512**
7) Krüüner 2003 &	5	clinical	54		*rrs* **A1401G**																12	42		
7) Krüüner 2003 &	5	*in vitro* mutants	4		*rrs* **A1401G**																	4		
7) Krüüner 2003 &	5	*in vitro* mutants	1		*rrs* **A1401G**																	1		
7) Krüüner 2003 &	5	*in vitro* mutants	2		*rrs* **A1401G**																	2		
7) Krüüner 2003 &	5	*in vitro* mutants	1		*rrs* **A1401G**																	1		
7) Krüüner 2003 &	5	*in vitro* mutants	5		*rrs* **A1401G**																1	4		
8) Pholwat 2011,	6	clinical	1	different levels of R	*rrs* **A1401G**									1										
13) Hu 2013	10	clinical	40		*rrs* **A1401G**													2	14		16		6	2
14) van Ingen 2010	11	clinical	5	MDR	*rrs* **A1401G**												5							
17) Via 2010	14	clinical	16		*rrs* **A1401G**															16				
18) Maus 2005a &	9	*in vitro* mutants	15	H37Rv or CDC1551	*rrs* **A1401G**															15				
18) Maus 2005a &	9	clinical	11		*rrs* **A1401G**															11				
19) Maus 2005b	9	clinical	13		*rrs* **A1401G**															13				
7) Krüüner 2003 &	5	*in vitro* mutants	4		*rrs* C1402T		1	2		1														
13) Hu 2013	10	clinical	2		*rrs* C1402T													2						
14) van Ingen 2010	11	clinical	3	MDR	*rrs* C1402T												3							
18) Maus 2005a &	9	*in vitro* mutants	5		*rrs* C1402T					5														
8) Pholwat 2011,	6	clinical	1	different levels of R	*rrs* **G1484T**									1										
18) Maus 2005a &	9	*in vitro* mutants	23		*rrs* **G1484T**										3			3	9	8				
18) Maus 2005a &	9	clinical	1		*rrs* **G1484T**															1				

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L). Notable limitations: Studies 18 and 19 were performed in the same laboratory. The genotypic results for Study 14 were based on the MTBDR*sl* v1.

eis mutants

Allelic exchange results

Zaunbrecher *et al.* (Study 12) tested all of the strains from their allelic exchange experiments (Section 2.A.2.2) against AMK (Table 23). Compared with the MIC of 0.5 mg/L for the parent strain, the introduction of *eis* G-10A, C-12T, A-13G, and G-37T mutations did not significantly affect the MICs. By contrast, the MIC of the C-14T mutant was increased by two doubling dilutions and was consequently susceptible at the current CC, but had an MIC above the lowered CC of 2 mg/L. This finding was confirmed by Pholwat *et al.* (Study 15), who observed an MIC of 4 mg/L for the C-14T mutation whereas the remaining *eis* mutants (i.e. G-10A, C-12T, C-14G, and C-15G) had MICs ≤2 mg/L.

Table 23. AMK MICs for eis allelic exchange mutants on 7H10.

					AMK MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**3**	**4**	**8**
12) Zaunbrecher 2009	allelic exchange mutants & complemented strains	1	H37Rv	gWT parent		**1**					
		2		*eis* **C-14T** introduced by alleleic exchange					2		
		1	H37Rv	*eis* **C-14T** *in vitro* parent					1		
		2		gWT (*eis* mutation removed by allelic exchange)		2					
		1	H37Rv Δ*eis*	complemented with WT *eis*		1					
		1		complemented with *eis* G-10A			1				
		1		complemented with *eis* C-12T			1				
		1		complemented with *eis* A-13G		1					
		1		complemented with *eis* **C-14T**				1			
		1		complemented with *eis* G-37T			1				
15) Pholwat 2016	allelic exchange mutants	1	H37Rv	gWT parent			**1**				
		1		gWT recombinant			1				
		3		*eis* G-10A introduced by alleleic exchange				3			
		3		*eis* C-12T introduced by alleleic exchange				3			
		3		*eis* C-14G introduced by alleleic exchange			3				
		3		*eis* **C-14T** introduced by alleleic exchange						3	
		2		*eis* C-15G introduced by alleleic exchange			2				

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L).

In vitro and clinical isolates

Three studies reported MIC data for 71 *in vitro* or clinical isolates which only had *eis* promoter mutations (Table 24). The MIC results were varied. At 32-64 mg/L, the MICs of five mutants from Hu *et al.* (Study 13) were unusually high, which may be due to the presence of other, un-identified resistance mutations. The sole C-14T mutant tested in Pholwat *et al.* (Study 15) had an MIC of 4 mg/L, comparable to the findings of the allelic exchange experiments. Similarly, the MIC distribution of 1-4 mg/L (with a mode at 2 mg/L) for this mutation was clearly elevated in Krüüner *et al.* & Engström *et al.* (Study 7) compared with the corresponding pWT distribution, with some overlap at the dataset-specific CC of 1 mg/L (i.e. *eis* C-14T behaved similarly to the *rrs* C1402T mutation). By contrast, the *eis* C-14T distribution from Hu *et al.* (Study 13) was systematically lower by one dilution (i.e. 0.5-1 mg/L), although it should be noted that the shape of the pWT MIC distribution was unclear in this study. There was no clear indication that any of the remaining *eis* mutations conferred elevated MICs.

Table 24. AMK MICs for in vitro and clinical eis mutants on 7H10.

				AMK MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
7) Krüüner 2003 & Engström 2011	clinical	35	*eis* G-10A		6	26	3						
13) Hu 2013	clinical	9	*eis* G-10A		1	4	1				2	1	
7) Krüüner 2003 & Engström 2011	clinical	2	*eis* C-12T			2							
7) Krüüner 2003 & Engström 2011	clinical	10	*eis* **C-14T**			2	5	3					
7) Krüüner 2003 & Engström 2011	*in vitro* mutants	1	*eis* **C-14T**		1								
7) Krüüner 2003 & Engström 2011	*in vitro* mutants	3	*eis* **C-14T**					3					
7) Krüüner 2003 & Engström 2011	*in vitro* mutants	1	*eis* **C-14T**				1						
13) Hu 2013	clinical	6	*eis* **C-14T**		1	2	1				2		
15) Pholwat 2016	clinical	1	*eis* **C-14T**					1					
7) Krüüner 2003 & Engström 2011	clinical	1	*eis* C-15G			1							
7) Krüüner 2003 & Engström 2011	clinical	2	*eis* G-37T			1	1						

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L).

whiB7 mutants

In vitro isolate

A single C+134 *whiB7 in vitro* mutant, which was resistant to KAN on 7H10 (Section 2.A.2.2), was susceptible to AMK on 7H10 at the current CC (Table 25).

Table 25. AMK MICs for in vitro whiB7 mutant on 7H10.

				AMK MIC (mg/L)				
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**4**	**8**	**16**	**32**	**64**
18) Maus 2005a & Reeves 2013	*in vitro* mutants	1	*whiB7* C+134	1				

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L).

tlyA mutants

In vitro and clinical isolates

All 38 *in vitro* or clinical *tlyA* mutants (100% (95% 91-100%)) tested in two laboratories were susceptible at the current CC (Table 26). Looking at the non-truncated data from Krüüner *et al.* & Engström *et al.* (Study 7), lowering the CC to 2 mg/L would not change this conclusion.

Table 26. AMK MICs for in vitro and clinical tlyA mutants on 7H10.

					AMK MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
19) Maus 2005b	9	clinical	4	*tlyA* mutants						4	
	5	clinical	4	*tlyA* mutants		1	3				
	5	*in vitro* mutants	8	*tlyA* mutants			3	5			
	5	*in vitro* mutants	5	*tlyA* mutants		1	4				
7) Krüüner 2003 & Engström 2011	5	*in vitro* mutants	2	*tlyA* mutants		1	1				
	5	*in vitro* mutants	3	*tlyA* mutants				3			
	5	*in vitro* mutants	2	*tlyA* mutants				2			
	5	*in vitro* mutants	2	*tlyA* mutants			1	1			
18) Maus 2005a & Reeves 2013	9	*in vitro* mutants	8	*tlyA* mutants						8	

The green line denotes the current WHO and CLSI CC for AMK DST on 7H10 (4 mg/L). Notable limitation: Studies 18 and 19 were performed in the same laboratory.

2.B.2.3 Conclusion for AMK CC on 7H10

On balance, the pWT MIC data supported **2 mg/L** as the CC for AMK on 7H10. The current CC of 4 mg/L was consequently lowered accordingly. The implications of this change for the interpretation of the *rrs* C1402T and *eis* C-14T mutations will be addressed at a later date.

2.B.3 AMK MIC data on 7H11

2.B.3.1 AMK MICs for pWT isolates on 7H11

Two studies were identified that reported AMK MIC data for the pWT population on 7H11, which were determined to be insufficient to propose a CC (Table 27).

Table 27. AMK MICs for pWT isolates on 7H11.

				AMK MIC (mg/L)											
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**0.25**	**0.5**	**0.62**	**1**	**1.25**	**2**	**2.5**	**4**	**5**	**8**	**16**	**32**	**64**
20) Fattorini 1999	clinical	1	H37Rv ATCC 27294						1							
		46	R to at least 2 first-line drugs		3		9		17		8		1			8
21) Rey-Jurado 2013a, Rey-Jurado 2013b & López-Gavín 2016	clinical	1	H37Rv							1						
		11	pan-S							11						
		9	MDR							9						

2.B.3.2 AMK MICs for mutated isolates on 7H11

No studies presenting MIC for the mutants were identified.

2.B.3.3 Conclusion for AMK CC on 7H11

A CC for AMK was not set for 7H11 given that only two studies were identified for this medium, and more evidence was available to set CCs for other media.

2.B.4 AMK MIC data in MGIT

2.B.4.1 AMK MICs for pWT isolates in MGIT

15 studies from 20 laboratories were identified that reported AMK MIC data for the pWT population in MGIT (Table 28). Most studies had a severely truncated pWT MIC distribution, including Rüsch-Gerdes *et al.* (Study 30) and Rodrigues *et al.* (Study 33), which have both been cited in the CLSI guidelines to support the current CC of 1 mg/L.[9] Where modes were identifiable, they varied between 0.25 to 1 mg/L, which supported the current CC.

2.B.4.2 AMK MICs for mutated isolates in MGIT

rrs mutants

Clinical isolates

Eight studies conducted in 13 laboratories were identified that interrogated 142 clinical isolates harbouring the *rrs* A1401G mutation (Table 29). All were resistant (100% (95% CI, 97-100%)) at the current CC.

Table 29. AMK MICs for clinical rrs mutants in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	AMK MIC (mg/L) 1	4	8	10	20	30	32	40	50	64	80
22) Gonzalo 2015	17	clinical	1	2		*rrs* **A1401G**				2							
23) Tessema 2017	18	clinical	3	3		*rrs* **A1401G**									3		
24) Heyckendorf 2017	18	clinical	4	4	MDR or XDR	*rrs* **A1401G**									4		
26) Zimenkov 2013	20	clinical	32	32		*rrs* **A1401G**											32
27) Kambli 2016a & 2016b	21	clinical	48	48		rrs **A1401G**									48		
34) Cambau 2015	4-5,11,18,27-31	clinical	18	18	MDR	*rrs* **A1401G**						18					
35) Sirgel 2012	32	clinical	35	35	MDR or XDR	*rrs* **A1401G**						35					
36) Springer 2009	4	clinical	1	1		*rrs* **A1401G**										1	

The green lines denote the current WHO and CLSI CCs for AMK DST in MGIT (1 mg/L). Notable limitations: Studies 23 and 24 and Studies 34 and 36 had data from the same laboratories. The genotypic results for Study 26 were based on a combination of sequencing and a microarray, whereas Study 27 relied on a combination of sequencing and the MTBDR*sl* v1.

eis mutants

Clinical isolates

Four studies from three laboratories presented data for clinical *eis* mutants (Table 30). All 34 mutants with *eis* mutations other than C-14T (100% (95% CI, 90-100%)) had MICs of ≤0.25-1 mg/L, and there was consequently no evidence that these mutations conferred elevated MICs to AMK. By contrast, there was a trend towards higher MICs for the C-14T mutants with 13 of the 17 MICs (77% (95% CI, 50-93%)) at 1-2 mg/L. In line with the 7H10 data (Section 2.B.2.2), this supported the hypothesis that this particular *eis* mutation conferred elevated AMK MICs.

9 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Table 28. AMK MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	AMK MIC (mg/L) 0.12	0.25	0.5	1	2	2.5	4	8	16	20	32	64	80	128	256	512	1024
22) Gonzalo 2015	17		1	2	H37Rv			1	1														
	17	clinical	20	40		gWT	1	20	19														
23) Tessema 2017	18	clinical	40	40		gWT			19	20			1										
24) Heyckendorf	18		1	1	H37Rv ATCC 27294				1														
2017	18	clinical	9	9	MDR or XDR	gWT		2	7														
25) Sturegård 2015	19		1	4	H37Rv ATCC 27294				4														
	19	clinical	28	28				8	12	3											1	1	3
26) Zimenkov 2013	20		1	1	H37Rv ATCC 25618					1													
	20	clinical	42	42		gWT		26	11	4									1				
27) Kambli 2016a &	21		1	1	H37Rv ATCC 27294			1															
2016b	21	clinical	31	31		gWT		25	5				1										
28) Matt 2012	4	clinical	10	10	pan-S			5		5													
29) Lin 2009	22		1	3	H37Rv ATCC 27294				3														
	23		1	14	H37Rv ATCC 27294				14														
	23	clinical	29	29						14	3	12											
30) Rüsch-Gerdes	18, 24-25	clinical	10	30	H37Rv ATCC 27294 & pan-S				30														
2006	18, 24-25		21	63	different levels of R				36	9	3	15											
31) Zheng 2016	10		1	1	H37Rv ATCC 25618				1														
	10	clinical	207	207	MDR				38	29	6		14	30	42		12	25		11			
32) Sharma 2011	26	clinical	36	36	different levels of R				22	4	1	9											
33) Rodrigues 2008	21	clinical	1	1	H37Rv ATCC 27294				1														
	21		10	10	pan-S				10														
	21		20	20	different levels of R				16	2	1	1											
34) Cambau 2015	4-5, 11, 18, 27-31	clinical	113	113	MDR					113													
	4-5, 11, 18, 27-31		3	3	MDR	gWT				1			2										
35) Sirgel 2012	32		1	1	H37Rv ATCC 27294					1													
	32	clinical	15	15	MDR or XDR	gWT				15													
36) Springer 2009	4	clinical	11	11		gWT				11													

The green line denotes the current WHO and CLSI CC for AMK DST in MGIT (1 mg/L). Notable limitations: Only Studies 22, 25, 26, 29, 31, 32 and 35 had data from unique laboratories. The genotypic results for Study 26 were based on a combination of sequencing and a microarray, whereas Study 27 relied on a combination of sequencing and the MTBDRs/ v1.

Table 30. AMK MICs for clinical eis mutants in MGIT.

						AMK MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
22) Gonzalo 2015	17	clinical	1	2	*eis* G-10A		2					
24) Heyckendorf 2017	18	clinical	3	3	*eis* G-10A		1	2				
26) Zimenkov 2013	20	clinical	10	10	*eis* G-10A	2	2	6				
27) Kambli 2016a & 2016b	21	clinical	3	3	*eis* G-10A		1	2				
27) Kambli 2016a & 2016b	21	clinical	3	3	*eis* G-10C	1	2					
23) Tessema 2017	18	clinical	1	1	*eis* C-12T			1				
26) Zimenkov 2013	20	clinical	2	2	*eis* C-12T		2					
27) Kambli 2016a & 2016b	21	clinical	2	2	*eis* C-12T	1	1					
22) Gonzalo 2015	17	clinical	3	6	*eis* **C-14T**			4	2			
23) Tessema 2017	18	clinical	1	1	*eis* **C-14T**		1					
26) Zimenkov 2013	20	clinical	7	7	*eis* **C-14T**	1		6				
27) Kambli 2016a & 2016b	21	clinical	3	3	*eis* **C-14T**	2		1				
22) Gonzalo 2015	17	clinical	1	2	*eis* G-37T		2					
23) Tessema 2017	18	clinical	1	1	*eis* G-37T			1				
24) Heyckendorf 2017	18	clinical	1	1	*eis* G-37T			1				
26) Zimenkov 2013	20	clinical	5	5	*eis* G-37T	1	1	3				
23) Tessema 2017	18	clinical	1	1	*eis* T-44C		1					

The green line denotes the current WHO and CLSI CCs for AMK DST in MGIT (1 mg/L). Notable limitations: Studies 23 and 24 had data from the same laboratory. The genotypic results for Study 26 were based on a combination of sequencing and a microarray, whereas Study 27 relied on a combination of sequencing and the MTBDR*sl* v1.

whiB7 mutants

Clinical isolate

The aforementioned *whiB7* A+238G mutation, which correlated with resistance to KAN on 7H10 but not MGIT (Sections 2.A.2.2 and 2.A.4.2), was susceptible to AMK (Table 31).

Table 31. AMK MICs for clinical whiB7 mutant in MGIT.

				AMK MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**1**	**4**	**20**	**40**
24) Heyckendorf 2017	clinical	1	*whiB7* A+238G			1				

The green line denotes the current WHO and CLSI CCs for AMK DST in MGIT (1 mg/L).

tlyA mutants

Clinical isolates

Two of the five clinical *tlyA* mutants (40% (95% CI, 5-85%)) from Cambau *et al.* (Study 34) were resistant at the current CC (Table 32). The two isolates that tested resistant had MICs one dilution above the CC, which may be due to the presence of other resistance mutations.

Table 32. AMK MICs for clinical tlyA mutants in MGIT.

					AMK MIC (mg/L)		
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**1**	**4**	**20**
34) Cambau 2015	clinical	5	MDR	*tlyA* mutants	3	2	

The green line denotes the current WHO and CLSI CCs for AMK DST in MGIT (1 mg/L).

2.B.4.3 Conclusion for AMK CC in MGIT

The CC of **1 mg/L** for AMK was reaffirmed.

2.B.5 References for AMK MIC studies

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6. Böttger, unpublished data.

7. (a) Krüüner, A., Juréen, P., Levina, K., Ghebremichael, S. & Hoffner, S. Discordant resistance to kanamycin and amikacin in drug-resistant *Mycobacterium tuberculosis. Antimicrob Agents Chemother* 47, 2971-3 (2003).

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2.C.1 CAP MIC data on LJ

2.C.1.1 CAP MICs for pWT isolates on LJ

Three studies from the same laboratory were identified that reported CAP MIC data for the pWT population on LJ (Table 33). Although the inter-laboratory reproducibility of testing could not be assessed, a mode at 20 mg/L was observed despite the truncation at the lower end of the pWT distribution.

Table 33. CAP MICs for pWT isolates on LJ.

						CAP MIC (mg/L)				
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**10**	**20**	**40**	**80**	**160**
1) Jugheli 2009	1	clinical	76		gWT	23	**39**	12	2	
2) Barletta 2014	1	clinical	7	MDR	gWT	4	3			
3) Vincent 2012	1	clinical	6	different levels of R	gWT	1	**3**	1	1	

The red line denotes the current WHO CC for CAP DST on LJ (40 mg/L). Notable limitation: All studies were performed in the same laboratory.

2.C.1.2 CAP MICs for mutated isolates on LJ

rrs mutants

Clinical isolates

Three studies from the same laboratory reported MICs for clinical *rrs* mutants (Table 34). All four *rrs* C1402T mutants (100% (95% 40-100%)) were resistant, with MICs of >160 mg/L. Six *rrs* A1401G mutants (8% (95% CI, 3-17%)) tested susceptible, which was mostly driven by the apparent overlap between the pWT distribution and the lower end of the MIC distribution of isolates with this mutation (i.e. it is likely that the five isolates with MICs of 40 mg/L would become resistant upon re-testing, whereas the isolate with an MIC of ≤10 mg/L might be the result of experimental error with either sequencing or pDST).

Table 34. CAP MICs for clinical rrs mutants on LJ.

						CAP MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**10**	**20**	**40**	**80**	**160**	**256**
1) Jugheli 2009	1	clinical	65		*rrs* **A1401G**	1		4	16	33	11
2) Barletta 2014	1	clinical	4	MDR	*rrs* **A1401G**					2	2
3) Vincent 2012	1	clinical	5	different levels of R	*rrs* **A1401G**			1		1	3
1) Jugheli 2009	1	clinical	4		*rrs* C1402T						4

The red line denotes the current WHO CC for CAP DST on LJ (40 mg/L). Notable limitation: All studies were performed in the same laboratory.

2.C.1.3 Conclusion for CAP CC on LJ

The identified CAP MIC data for LJ were limited and provided little insight into the shape of the pWT distribution, which precluded a re-assessment of the current CC. Nevertheless, **40 mg/L** was maintained as the CC, given the importance of LJ as a testing medium in many settings. An overlap between the MIC distributions of pWT isolates and *rrs* A1401G mutants was nonetheless noted.

2.C.2 CAP MIC data on 7H10

2.C.2.1 CAP MICs for pWT isolates on 7H10

11 studies from nine laboratories were identified that reported CAP MIC data for the pWT population on 7H10 (Table 35). Most distributions had modes at 1.25 or 2 mg/L, for which the WHO CC of 4 mg/L would be the optimal CC (e.g. for Krüüner *et al.* & Engström *et al.* (Study 6)). By contrast, Studies 9-11 had modes at either 4 or 5 mg/L. Among these, Reeves *et al.* (Study 9) was notable given that it presented data from the CDC, which uses 7H10 for routine pDST. The MIC distribution for the 16 isolates in this study was symmetrically centred around 4 mg/L, which meant that the use of the WHO CC would result in a systematic false-resistance rate of 13% (95% CI, 2-38%), assuming that these isolates are pWT. This would not be the case at the current CLSI CC of 10 mg/L or the equivalent concentration of 8 mg/L. For the pWT distribution in van Ingen *et al.* (Study 11), which consisted of 20 isolates, a false-resistance rate of 5% (95% CI, 0-25%) would occur at the WHO CC. However, it should be noted that relatively few isolates were tested as part of Studies 7 and 11 and it was therefore unclear whether these results are reproducible.

2.C.2.2 CAP MICs for mutated isolates on 7H10

rrs mutants

Allelic exchange results

Reeves *et al.* (Study 9) conducted allelic exchange experiments in H37Rv, CDC1551 and Beijing strain backgrounds (Table 36). The MICs of the three parent strains were 2-4 mg/L on 7H10, compared to 40 mg/L for *rrs* A1401G, 80 mg/L for *rrs* C1402T, and 160 to >320 mg/L for *rrs* G1484T.

Table 36. CAP MICs for rrs allelic exchange mutants on 7H10.

					CAP MIC (mg/L)											
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**1**	**2**	**4**	**6**	**8**	**10**	**14**	**20**	**40**	**80**	**160**	**320**	**512**
9) Reeves 2015	allelic exchange mutants	1	H37Rv	gWT		1											
		1		*rrs* **A1401G**									1				
		1		*rrs* C1402T										1			
		1		*rrs* **G1484T**													1
		1	CDC1551	gWT			**1**										
		1		*rrs* **A1401G**									1				
		1		*rrs* C1402T										1			
		1		*rrs* **G1484T**													1
		1	Beijing	gWT			**1**										
		1		*rrs* **A1401G**									1				
		1		*rrs* C1402T										1			
		1		*rrs* **G1484T**											1		

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).

Table 35. CAP MICs for pWT isolates on 7H10.

							CAP MIC (mg/L)																				
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.31**	**0.5**	**0.62**	**1**	**1.25**	**2**	**2.5**	**4**	**5**	**6**	**8**	**10**	**14**	**16**	**20**	**32**	**40**	**64**	**80**	**160**
4) Juréen 2010	2		1	4	H37Rv ATCC 27294						**3**		1														
	2	clinical	100	100	mostly pan-S						35		**60**		2			2					1				
5) Böttger	3		1	3	H37Rv ATCC 27294						1		**2**														
	3		1	3	Erdman ATCC 35801						1		**2**														
	3	clinical	84	84	pan-S or non-MDR				1		11		**67**		5												
6) Křüüner 2003 & Engström 2011	4	clinical	42	42		gWT	1		4		17		**19**		1												
	4		1	1	H37Rv ATCC 25618	gWT parent					**1**																
	4		1	1	H37Ra ATCC 25177	gWT parent			**1**																		
	4		1	1	E55	gWT parent							**1**														
	4		1	1	E3942	gWT parent					**1**																
	4		1	1	BTB 09-070	gWT parent							**1**														
	4		1	1	BTB 09-072	gWT parent							**1**														
	4		1	1	BTB 09-116	gWT parent					**1**																
7) Pholwat 2011, 2012 & 2015	5		1	1	H37Rv ATCC 27294									**1**													
	5	clinical	35	35	different levels of R	gWT				7		**22**		2		4											
8) Hu 2013	6		1	1	H37Rv ATCC 25618						**1**																
9) Reeves 2015	7		1	1	H37Rv	gWT							**1**														
	7		1	1	CDC1551	gWT									**1**												
	7		1	1	Beijing	gWT									**1**												
	7	clinical	19	19		gWT							2		**12**			2				1		2			
10) Sowajassatakul 2014	8		1	3	H37Rv ATCC 27294										**3**												
	8	clinical	27	81		gWT							30		**51**												
	8		3	6		gWT															6						
11) van Ingen 2010	9		1	1	H37Rv ATCC 27294								1														
	9	clinical	20	20	MDR	gWT							4			**15**			1								
12) Via 2010	10	clinical	90	90		gWT													88					1			1
13) Maus 2005a,	7	clinical	4	4		gWT																2				2	
14) Maus 2005b & Johansen 2006	7		1	1	H37Rv	gWT parent													1								
	7		1	1	CDC1551	gWT parent													1								
	7		1	1	Beijing F2	gWT parent													1								
	7		1	1	Beijing D3	gWT parent													1								

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).
Notable limitations: Studies 9, 13 and 14 were done in the same laboratory. The genotypic results for Study 11 were based on the MTBDR*s*/ v1.

In vitro and clinical isolates

Nine studies from seven laboratories reported MICs for *in vitro* or clinical *rrs* mutants (Table 37). All 41 *rrs* C1402T and G1484T mutants were resistant (100% (95% CI, 91-100%)) at the current WHO CC. By contrast, the detection of *rrs* A1401G was compromised by the variation between different laboratories and/or different datasets and the fact that the distributions of these isolates overlapped with the upper end of the pWT distributions. For example, Krüüner *et al.* & Engström *et al.* (Study 6) had systematically lower pWT MIC distributions and, consequently, a dataset-specific CC of 4 mg/L, which also represented the lower end of the *rrs* A1401G distribution (Section 2.C.2.1). By contrast, Reeves *et al.* (Study 9) and van Ingen *et al.* (Study 10) both had higher pWT MIC distributions and dataset-specific CCs of 8 and 10 mg/L, which were also the lower ends of the respective *rrs* A1401G distributions of these studies. Although all 242 *rrs* A1401G mutants likely had elevated MICs (100% (95% CI, 98-100%)), different percentages of these mutants would be misclassified depending on which CC was used. Using the WHO CC of 4 mg/L, only four isolates (2% (95% CI, 0-4%)) were misclassified as susceptible, whereas this increased to 56 (23% (95% CI, 18-29%)) with the CLSI CC of 10 mg/L.

eis mutants

In vitro and clinical isolates

CAP MICs were reported for 73 *eis* mutants in four studies (Table 38). Excluding one isolate from Study 13, for which the lowest concentration tested was 10 mg/L, six isolates (8% (95% CI, 3-17%)) were resistant at the WHO CC, which decreased to five (7% (95% CI, 2-15%)) at the CLSI CC. The remaining five isolates had unusually high MICs (32-64 mg/L), likely due to the presence of another resistance mutation.

Table 38. CAP MICs for in vitro and clinical eis mutants on 7H10.

					CAP MIC (mg/L)												
Studies	Isolate origin	Unique isolates	Total MICs	Genotypic results	0.25	0.5	1	2	4	8	10	16	20	32	40	64	80
6) Krüüner 2003 & Engström 2011	clinical	35	35	*eis* G-10A			11	20	4								
8) Hu 2013	clinical	9	9	*eis* G-10A			1	3	2					3			
6) Krüüner 2003 & Engström 2011	clinical	2	2	*eis* C-12T				2									
6) Krüüner 2003 & Engström 2011	clinical	10	10	*eis* **C-14T**			1	5	4								
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	1	1	*eis* **C-14T**		1											
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	3	3	*eis* **C-14T**					3								
6) Krüüner 2003 & Engström 2011	*in vitro* mutants	1	1	*eis* **C-14T**				1									
8) Hu 2013	clinical	6	6	*eis* **C-14T**			1	1	2					1		1	
10) Sowajassatakul 2014	clinical	1	2	*eis* **C-14T**						2							
13) Maus 2005a, Zaunbrecher 2009	*in vitro* mutants	1	1	*eis* **C-14T**							1						
6) Krüüner 2003 & Engström 2011	clinical	1	1	*eis* C-15G				1									
6) Krüüner 2003 & Engström 2011	clinical	2	2	*eis* G-37T			1	1									
10) Sowajassatakul 2014	clinical	1	2	*eis* G-37T													2

The red lines denote the current WHO CCs for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).

whiB7 mutants

In vitro isolate

One *in vitro whiB7* mutant, which was resistant to KAN on 7H10 (Section 2.A.2.2), was susceptible to CAP at the CLSI CC of 10 mg/L (Table 39).

Table 39. CAP MICs for in vitro whiB7 mutant on 7H10.

				CAP MIC (mg/L)						
Studies	Isolate origin	Unique isolates	Genotypic results	4	8	10	20	40	80	160
13) Maus 2005a, Zaunbrecher 2009	*in vitro* mutants	1	*whiB7* C+134			1				

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).

Table 37. CAP MICs for in vitro and clinical rrs mutants on 7H10.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	CAP MIC (mg/L) 2	4	5	8	10	12.5	14	16	20	32	40	64	80	128	160	256	320	512
6) Krüüner 2003 & Engström 2011	4	clinical	54	54		*rrs* **A1401G**		3		35				11		5								
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	4	4		*rrs* **A1401G**								4										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	1	1		*rrs* **A1401G**								1										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	2		*rrs* **A1401G**								2										
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	1	1		*rrs* **A1401G**										1								
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	5	5		*rrs* **A1401G**				2				3										
7) Pholwat 2011, 2012 & 2015	5	clinical	1	1	different levels of R	*rrs* **A1401G**						1												
8) Hu 2013	6	clinical	40	40		*rrs* **A1401G**				3				6		5		16		10				
9) Reeves 2015	7	clinical	53	53		*rrs* **A1401G**				8			4		14		27							
10) Sowajassatakul 2014	8	clinical	21	42		*rrs* **A1401G**		2		2				12		24			2					
11) van Ingen 2010	9	clinical	5	5	MDR	*rrs* **A1401G**					1				2		2							
12) Via 2010	10	clinical	16	16		*rrs* **A1401G**									6		6		2		2			
13) Maus 2005a, Zaunbrecher 2009	7	*in vitro* mutants	15	15	H37Rv or CDC1551	*rrs* **A1401G**									5		8		1			1		
13) Maus 2005a, Zaunbrecher 2009	7	clinical	11	11		*rrs* **A1401G**									8		2		1					
14) Maus 2005b & Johansen 2006	7	clinical	13	13		*rrs* **A1401G**									4		2		2			5		
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	4	4		*rrs* C1402T													4					
8) Hu 2013	6	clinical	2	2		*rrs* C1402T												2						
9) Reeves 2015	7	clinical	1	1		*rrs* C1402T											1							
11) van Ingen 2010	9	clinical	3	3	MDR	*rrs* C1402T									1		1		1					
13) Maus 2005a, Zaunbrecher 2009	7	*in vitro* mutants	5	5		*rrs* C1402T															4	1		
7) Pholwat 2011, 2012 & 2015	5	clinical	1	1	different levels of R	*rrs* **G1484T**						1												
9) Reeves 2015	7	clinical	1	1		*rrs* **G1484T**																		1
13) Maus 2005a, Zaunbrecher 2009	7	*in vitro* mutants	23	23		*rrs* **G1484T**																23		
13) Maus 2005a, Zaunbrecher 2009	7	clinical	1	1		*rrs* **G1484T**																1		

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).
Notable limitations: Studies 9, 13 and 14 were done in the same laboratory. The genotypic results for Study 11 were based on the MTBDRs*l* v1.

tlyA mutants

In vitro and clinical isolates

Three studies from two laboratories reported MICs for 48 *in vitro* and clinical *tlyA* mutants, of which 92% (95% CI, 80-98%) were resistant at both CCs (Table 40). Two clinical mutants had very low MICs (i.e. 0.5 and 1 mg/L), which means that the mutations in question could be genuine polymorphisms that do not confer resistance. Two *in vitro* mutants from Krüüner *et al.* & Engström *et al.* (Study 6) had MICs of 8 mg/L, which were elevated compared with the dataset-specific CC of 4 mg/L for this study.

Table 40. CAP MICs for in vitro and clinical tlyA mutants on 7H10.

					CAP MIC (mg/L)													
Studies	Lab	Isolate origin	Unique isolates	Genotypic results	0.25	0.5	1	2	4	8	10	16	20	32	40	64	80	160
6) Krüüner 2003 & Engström 2011	4	clinical	4	*tlyA* mutants		1	1					2						
14) Maus 2005b & Johansen 2006	7	clinical	4	*tlyA* mutants											1		3	
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	8	*tlyA* mutants								4		4				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	5	*tlyA* mutants						2		2		1				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants								2						
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	3	*tlyA* mutants										3				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants										2				
6) Krüüner 2003 & Engström 2011	4	*in vitro* mutants	2	*tlyA* mutants								2						
13) Maus 2005a, Zaunbrecher 2009	7	*in vitro* mutants	8	*tlyA* mutants											4		3	1
14) Maus 2005b & Johansen 2006	7	*in vitro* mutants	4	*tlyA* mutants											4			
14) Maus 2005b & Johansen 2006	7	*in vitro* mutants	3	*tlyA* mutants											3			
14) Maus 2005b & Johansen 2006	7	*in vitro* mutants	3	*tlyA* mutants											3			

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L). Notable limitation: Studies 13 and 14 were done in the same laboratory.

rrl mutant

In vitro isolate

Maus *et al.* & Johansen *et al.* (Study 14) reported a CAP MIC of >160 mg/L for an *in vitro rrl* mutant (Table 41). This is the only *rrl* mutation implicated in CAP resistance to date. The authors reported that this mutant remained susceptible to both KAN and AMK.

Table 41. CAP MICs for in vitro rrl mutant on 7H10.

				CAP MIC (mg/L)							
Studies	Isolate origin	Unique isolates	Genotypic results	4	8	10	20	40	80	160	256
14) Maus 2005b & Johansen 2006	*in vitro* mutants	1	gWT parent			1					
		1	*rrl* Δ1916								1

The red line denotes the current WHO CC for CAP DST on 7H10 (4 mg/L). The blue line denotes the current CLSI CC for CAP DST on 7H10 (10 mg/L).

2.C.2.3 Conclusion for CAP CC on 7H10

The available data supported **4 mg/L** as the current CC for CAP DST on 7H10. However, it should be noted that the modes of two of the pWT distributions were 4 mg/L, which may mean that the gain in sensitivity in detecting pNWT isolates may come at the expense of a higher rate of false-resistance detection. However, if the CC was raised to 8 mg/L, there would be a greater risk that strains with the *rrs* A1401G mutation may be misclassified as susceptible. As more laboratories adopt 4 mg/L as the CC for pDST, both these possibilities should be monitored.

2.C.3 CAP MIC data on 7H11

2.C.3.1 CAP MICs for pWT isolates on 7H11

Only Fattorini *et al.* (Study 14) reported CAP MIC data for 7H11 (Table 42).

Table 42. CAP MICs for pWT isolates on 7H11.

				CAP MIC (mg/L)								
Studies	Isolate origin	Unique isolates	Type of isolates	0.31	0.62	1.25	2.5	5	10	20	40	80
15) Fattorini 1999		1	H37Rv ATCC 27294				**1**					
	clinical	46	R to at least 2 first-line drugs		1	4	10	**21**	5	4	1	

The blue line denotes the current CLSI CC for CAP DST on 7H11 (10 mg/L).

2.C.3.2 CAP MICs for mutated isolates on 7H11

No studies presenting MIC data for isolates with mutations in resistance genes were identified.

2.C.3.3 Conclusion for CAP CC on 7H11

Given that only a single study was identified for 7H11, which precluded an assessment of the inter-laboratory reproducibility, a CC for this medium could not be set.

2.C.4 CAP MIC data in MGIT

2.C.4.1 CAP MICs for pWT isolates in MGIT

13 studies from 19 laboratories featured CAP MIC data for the pWT population in MGIT (Table 43). Several of the distributions were severely truncated, including those for Rüsch-Gerdes *et al.* (Study 22) and Rodrigues *et al.* (Study 24), which were cited by CLSI in support of the current CC of 2.5 mg/L.[10] Moreover, different dilution series were used by most of the studies, which complicated the direct comparison of the MICs. Nevertheless, the current CC appeared to be appropriate for the majority of studies, although there is at least one exception (i.e. Sturegård *et al.* (Study 16) would be better served by a CC of 4 mg/L, but additional untruncated MIC distributions, generated using standard dilution series, are required to investigate this possibility).

2.C.4.2 CAP MICs for mutated isolates in MGIT

rrs mutants

Clinical isolates

Seven studies reported MIC data from 153 clinical *rrs* A1401G mutants from 12 laboratories (Table 44). Unlike LJ and 7H10, there did not appear to be an overlap between the pWT MIC distribution and the mutant MIC distribution in MGIT, as the lower end of the distribution of these mutants was 5 mg/L, so none of the isolates were misclassified (0% (95% CI, 0-2%)).

Table 44. CAP MICs for clinical** rrs **mutants in MGIT.

						CAP MIC (mg/L)							
Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic results	1.25	2.5	5	10	12.5	15	20	25
17) Tessema 2017	13	clinical	3		*rrs* **A1401G**			2		1			
18) Heyckendorf 2017	13	clinical	4	MDR or XDR	*rrs* **A1401G**			3		1			
19) Gikalo 2012 & Zimenkov 2013	14	clinical	32		*rrs* **A1401G**			18	13			1	
23) Kambli 2016a & 2016b	19	clinical	48		*rrs* **A1401G**			25	20		3		
26) Pietersen 2015	21	clinical	13	XDR	*rrs* **A1401G**			9	3	1			
28) Cambau 2015	3-4, 9, 13, 22-26	clinical	18	MDR	*rrs* **A1401G**			3					15
29) Sirgel 2012	21	clinical	35	MDR or XDR	*rrs* **A1401G**				31		4		

The green line denotes the current WHO and CLSI CC for CAP DST in MGIT (2.5 mg/L). Notable limitations: Studies 17, 18 and 28, and Studies 26 and 29 reported data from the same laboratory, respectively. The genotypic results for Study 19 were based on a combination of sequencing and a microarray, whereas Study 23 relied on a combination of sequencing and the MTBDR*sl* v1.

10 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Table 43. CAP MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	CAP MIC (mg/L) 0.31	0.5	0.62	1	1.25	1.5	2	2.5	3	4	5	6	8	10	12.5	15	16	25	32	64	128
16) Sturegård 2015	12		1	3	H37Rv ATCC 27294								**3**														
	12	clinical	20	20						4			**12**			3			1								
17) Tessema 2017	13	clinical	40	40		gWT			1		**24**			14			1										
18) Heyckendorf	13		1	1	H37Rv ATCC 27294						**1**																
2017	13	clinical	9	9	MDR or XDR	gWT					**9**																
19) Gikalo 2012 &	14		1	1	H37Rv ATCC 25618									**1**													
Zimenkov 2013	14	clinical	33	33		gWT	2		3		**25**			2						1							
20) Zheng 2016	6		1	1	H37Rv ATCC 25618					**1**																	
	6	clinical	207	207	MDR			57		41			**68**						4				7		12	15	3
21) Lin 2009	15		1	3	H37Rv ATCC 27294					**3**																	
	16		1	15	H37Rv ATCC 27294					5	3	**7**															
	16	clinical	29	29									14			10	5										
22) Rüsch-Gerdes	13, 17-18	clinical	10	30	H37Rv ATCC 27294 & pan-S				10		**19**			1													
2006	13, 17-18		21	63	different degrees of R				11		**31**				21												
23) Kambli 2016a &	19		1	1	H37Rv ATCC 27294				1																		
2016b	19	clinical	31	31		gWT			25		5						1										
	19		1	1	H37Rv ATCC 27294				1																		
24) Rodrigues 2008	19	clinical	10	10	pan-S				1		**5**			4													
	19		20	20	differnt levels or R				4		**14**			1	1												
25) Sharma 2011	20	clinical	36	36	different levels of R						19			5			1	11									
27) Springer 2009	3	clinical	12	12		gWT					9						3										
28) Cambau 2015	3-4, 9, 13, 22-26	clinical	113	113	MDR									113													
	3-4, 9, 13, 22-26		3	3	MDR	gWT								2										1			
29) Sirgel 2012	21		1	1	H37Rv ATCC 27294									1													
	21	clinical	15	15	MDR or XDR	gWT								15													

The green line denotes the current WHO and CLSI CC for CAP DST in MGIT (2.5 mg/L). Notable limitations: Only studies 16, 19-21, 25 and 29 had data from unique laboratories. The genotypic results for Study 19 were based on a combination of sequencing and a microarray, whereas Study 23 relied on a combination of sequencing and the MTBDRs*l* v1.

eis mutants

Clinical isolates

All 43 *eis* mutants tested (100% (95% CI, 92-100%)) in three different laboratories were susceptible at the current CC (Table 45).

Table 45. CAP MICs for clinical eis mutants in MGIT.

						CAP MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.31**	**0.62**	**1.25**	**2.5**	**5**	**10**	**12.5**
18) Heyckendorf 2017	13	clinical	3	MDR or XDR	*eis* G-10A			3				
19) Gikalo 2012 & Zimenkov 2013	14	clinical	10		*eis* G-10A		1	5	4			
23) Kambli 2016a & 2016b	19	clinical	3		*eis* G-10A		1	1	1			
23) Kambli 2016a & 2016b	19	clinical	3		*eis* G-10C		3					
17) Tessema 2017	13	clinical	1		*eis* C-12T				1			
19) Gikalo 2012 & Zimenkov 2013	14	clinical	2		*eis* C-12T			2				
23) Kambli 2016a & 2016b	19	clinical	2		*eis* C-12T		2					
17) Tessema 2017	13	clinical	1		*eis* **C-14T**			1				
19) Gikalo 2012 & Zimenkov 2013	14	clinical	7		*eis* **C-14T**			7				
23) Kambli 2016a & 2016b	19	clinical	3		*eis* **C-14T**		2	1				
17) Tessema 2017	13	clinical	1		*eis* G-37T				1			
18) Heyckendorf 2017	13	clinical	1	MDR or XDR	*eis* G-37T			1				
19) Gikalo 2012 & Zimenkov 2013	14	clinical	5		*eis* G-37T			4	1			
17) Tessema 2017	13	clinical	1		*eis* T-44C			1				

The green line denotes the current WHO and CLSI CC for CAP DST in MGIT (2.5 mg/L). Notable limitations: Studies 17 and 18 reported data from the same laboratory. The genotypic results for Study 19 were based on a combination of sequencing and a microarray, whereas Study 23 relied on a combination of sequencing and the MTBDRs*l* v1.

whiB7 mutant

Clinical isolates

The aforementioned *whiB7* A+238G mutation, which was correlated with KAN resistance on 7H10 but not MGIT (Sections 2.A.2.2 and 2.A.4.2), was susceptible to CAP (Table 46).

Table 46. CAP MICs for clinical whiB7 mutant in MGIT.

					CAP MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.31**	**0.62**	**1.25**	**2.5**	**5**	**12.5**	**25**
18) Heyckendorf 2017	clinical	1	MDR or XDR	*whiB7* A+238G			1				

The green line denotes the current WHO and CLSI CC for CAP DST in MGIT (2.5 mg/L).

tlyA mutant

Clinical isolates

Five clinical *tlyA* mutations all tested CAP-resistant (100% (95% CI, 48-100%)) at the current CC (Table 47).

Table 47. CAP MICs for clinical tlyA mutants in MGIT.

					CAP MIC (mg/L)		
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**2.5**	**5**	**25**
28) Cambau 2015	clinical	5	MDR	*tlyA* mutants		1	4

The green line denotes the current WHO and CLSI CC for CAP DST in MGIT (2.5 mg/L).

2.C.4.3 Conclusion for CAP CC in MGIT

The CC of **2.5 mg/L** for CAP was reaffirmed. Unlike LJ and 7H10, no overlap of the MIC distributions of pWT isolates and *rrs* A1401G mutants was observed.

2.C.5 References for CAP MIC studies

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3. Clofazimine and bedaquiline

3.0 CFZ and BDQ MIC data stratification and current breakpoints

MIC data were stratified by mutations in *atpE* (Rv1305), *pepQ* (Rv2535c) and *mmpR* (Rv0678) but not *Rv1979c*, given the substantial mutation variability observed for this gene (details regarding these resistance mechanisms can be found in the supplement). *mmpR* promoter mutations were assumed not to increase BDQ MICs, and so were shown as gWT in tables and data files.[11] For BDQ, LOF mutations in *mmpR* were highlighted whenever possible. Additionally, some studies tested BDQ concentrations of 0.24, 0.48 and 0.96 mg/L, which were normalised to 0.25, 0.5 and 1 mg/L in order to best collate the data.

No breakpoints for BDQ testing have been defined by CLSI, FDA or WHO to date. In parallel with the marketing authorisation by European Medicines Agency, EUCAST has designated 0.25 mg/L as a medium-independent CB for BDQ testing.[12] No CC for CFZ currently exists.

3.A.1 CFZ MIC data on LJ

3.A.1.1 CFZ MICs for pWT isolates on LJ

No studies with MICs for pWT isolates were identified.

3.A.1.2 CFZ MICs for mutated isolates on LJ

No studies were found.

3.A.1.3 Conclusion for CFZ CC for LJ

Given that no MIC data were identified for LJ, a CC could not be defined.

3.A.2 CFZ MIC data on 7H10

3.A.2.1 CFZ MICs for pWT isolates on 7H10

Two studies from two laboratories were identified that reported CFZ MIC data for the pWT population on 7H10 (Table 48). van Ingen *et al.* (Study 2) had a severely truncated MIC distribution, which precluded an assessment of its shape.

Table 48. CFZ MICs for pWT isolates on 7H10.

					CFZ MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**
1) Schön 2011		1	2	H37Rv ATCC 27294			**2**				
	clinical	64	64	mostly pan-S		1	21	**41**	1		
2) van Ingen 2010		1	1	H37Rv ATCC 27294					1		
	clinical	28	28	MDR					25	3	

3.A.2.2 CFZ MICs for mutated isolates on 7H10

No studies presenting MIC distributions for mutated isolates were found.

3.A.2.3 Conclusion for CFZ CC for 7H10

Only two studies were identified that reported CFZ MICs for 7H10. These data were insufficient to establish a CC.

11 Villellas, C. *et al.* Unexpected high prevalence of resistance-associated *Rv0678* variants in MDR-TB patients without documented prior use of clofazimine or bedaquiline. *J Antimicrob Chemother* 72, 684-690 (2017).
12 European Committee for Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, valid from 2017-03-10. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.xls (accessed 9.4.2017).

3.A.3 CFZ MIC data on 7H11

3.A.3.1 CFZ MICs for pWT isolates on 7H11

A single study was identified with CFZ MIC data for 7H11, which did not include H37Rv (Table 49).

Table 49. CFZ MICs for pWT isolates on 7H11.

Studies	Isolate origin	Unique isolates	Type of isolates	CFZ MIC (mg/L) 0.06	0.12	0.25	0.5	1
3) López-Gavîn 2015	clinical	11	pan-S		**11**			
		7	MDR		**7**			

3.A.3.2 CFZ MICs for mutated isolates on 7H11

No studies with MIC distributions for gNWT isolates on 7H11 were found.

3.A.3.3 Conclusion for CFZ CC for 7H11

Only a single study was identified that reported CFZ MICs on 7H11. Therefore, a CC was not set for this medium.

3.A.4 CFZ MIC data in MGIT

3.A.4.1 CFZ MICs for pWT isolates in MGIT

Six studies from nine laboratories reported CFZ MIC data for the pWT population in MGIT (Table 50). Despite the fact that most distributions were truncated, variation in testing was apparent, as the modes of the pWT MIC distribution varied between 0.25 and 1 mg/L. These data therefore suggested a CC of 1 mg/L, although yet unknown resistance mechanisms for this drug may have confounded this analysis.

Table 50. CFZ MICs for pWT isolates in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	CFZ MIC (mg/L) 0.06	0.12	0.25	0.5	1	2	4	5
4) Köser	4	clinical	1	21	H37Rv ATCC 9360			9	**12**					
	4		16	16	pan-S			1	**12**	3				
	4		17	17	different levels of R			3	**10**	4				
5) van Ingen 2010	2		1	1	H37Rv ATCC 27294			1						
	2	clinical	28	28	MDR			11	**15**	2				
6) Dheda 2017	5	clinical	148	148	XDR			8	44	**95**	1			
7) Ismail	6	clinical	1	3	H37Rv				1	**2**				
	6		9	9	pan-S				7	2				
	6		2	2	MDR & XDR					**1**	**1**			
	6		34	34	MDR & XDR	gWT			12	**16**	4	1		1
	6		1	1	H37Rv					**1**				
	7		1	3	H37Rv				2	1				
	7		9	9	pan-S				1	**8**				
	7		19	19	mostly MDR or XDR				8	6	5			
	7		25	25	mostly MDR or XDR	gWT			9	**13**	3			
	7		1	1	H37Rv				1					
	8		1	1	H37Rv					**1**				
	8	clinical	44	44	MDR				3	**33**	5	3		
	8		1	1	H37Rv					**1**				
	9	clinical	11	11		gWT			6	4	1			
	9		1	1	H37Rv				1					
8) Bloemberg 2015,	10	clinical	27	27	pan-S					9	**18**			
Somoskovi 2015 & Böttger	10		1	1	pre-XDR	gWT					**1**			
9) Werngren	11	clinical	38	38	mostly MDR					38				

3.A.4.2 CFZ MICs for mutated isolates in MGIT

In vitro and clinical isolates

Five studies from eight laboratories presented MICs for *in vitro* and clinical mutants (Table 51). Köser *et al.* (Study 4) reported a low CFZ MIC of 0.12 mg/L for a BDQ-resistant, *in vitro atpE* mutant. The same set of *mmpR in vitro* mutants were tested in four laboratories by Ismail *et al.* (Study 7). Some of these mutants were also tested in a fifth laboratory by Köser *et al.* (Study 4), along with further *mmpR in vitro* mutants. The 62 MICs from these five laboratories varied between 1 and >4 mg/L, of which 32 were >1 mg/L (52% (95% CI, 39-65%)). By contrast, the MICs for the 17 clinical *mmpR* mutants ranged from ≤0.25 to >4 mg/L. This was likely due to the fact that some of these mutations negatively affected the function of *MmpR* and thus conferred elevated MICs, whereas others did not. For example, the *mmpR* V3I mutation, which was observed in seven isolates and was tested in two laboratories, always had MICs ≤0.5 mg/L and was therefore likely a neutral polymorphism. By contrast, the *mmpR* V1A mutation, which evolved during treatment with CFZ and correlated with cross-resistance to BDQ (Section 3.B.4.2), was significant, as it correlated with a MIC of ≥4 mg/L compared with 1 mg/L for the gWT parent.

Table 51. CFZ MICs for in vitro and clinical atpE and mmpR mutants in MGIT.

							CFZ MIC (mg/L)							
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**5**
4) Köser	4	*in vitro* mutants	1	1		*atpE* mutant		1						
	4		2	19		*mmpR* (2 different mut.)					13	6		
	4		11	11		*mmpR* (6 different mut.)						8	3	
7) Ismail	6	*in vitro* mutants	8	8		*mmpR* (7 different mut.)					5	2	1	
	7		8	8		*mmpR* (7 different mut.)					6	1	1	
	8		8	8		*mmpR* (7 different mut.)					1	4	3	
	9		8	8		*mmpR* (7 different mut.)					5	1	1	1
	6	clinical	4	4	MDR & XDR	*mmpR* V3I			3	1				
	6		6	6	MDR & XDR	*mmpR* mutants					2	2	2	
	7		3	3	mostly MDR or XDR	*mmpR* V3I			3					
	9		8	8		*mmpR* mutants			1	1	2	2		2
6) Dheda 2017	5	clinical	1	1	XDR	*mmpR* mutant					1			
8) Bloemberg 2015,	10		1	1	pre-XDR	gWT parent					**1**			
Somoskovi 2015 &	10		1	4	pre-XDR & XDR	*mmpR* V1A							3	1
9) Werngren	11	clinical	1	1	MDR	*mmpR* mutant						1		

3.A.4.3 Conclusion for CFZ CC for MGIT

Because of the variation in testing, an interim CC of **1 mg/L** was adopted. However, this concentration also corresponded to the lower end of the MIC distribution for *mmpR in vitro* isolates, which meant that the reproducibility of *mmpR* mutants that have truly elevated MICs would likely be poor (i.e. a large proportion would be misclassified as susceptible). In addition, Ismail *et al.* (Study 7) reported that the use of DMSO was crucial to prepare the inoculum and the dilutions for CFZ testing to prevent the precipitation of CFZ and, consequently, artificially high MICs (i.e. false-resistance). Therefore, more data for untruncated MIC distributions for pan-susceptible isolates are needed to better define the upper end of the pWT MIC distribution and the inter-laboratory reproducibility. Finally, additional studies of the various CFZ resistance mechanisms and their clinical relevance are needed.

3.A.5 References for CFZ MIC studies

1. Schön, T. *et al.* Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis. Int J Tuberc Lung Dis* 15, 502-9 (2011).

2. Van Ingen, J. *et al.* Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol* 48, 2749-53 (2010).

3. López-Gavín, A., Tudó, G., Vergara, A., Hurtado, J.C. & Gonzalez-Martín, J. *In vitro* activity against *Mycobacterium tuberculosis* of levofloxacin, moxifloxacin and UB-8902 in combination with clofazimine and pretomanid.

Int J Antimicrob Agents 46, 582-5 (2015).

4. Köser, unpublished data.

5. Van Ingen, J. *et al.* Comparative study on genotypic and phenotypic second-line drug resistance testing of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol* 48, 2749-53 (2010).

6. Dheda, K. *et al.* Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respir Med* 5, 269-281 (2017).

7. Ismail, unpublished data.

8. (a) Bloemberg, G.V. *et al.* Acquired resistance to bedaquiline and delamanid in therapy for tuberculosis. *N Engl J Med* 373, 1986-8 (2015).

(b) Somoskovi, A., Bruderer, V., Hömke, R., Bloemberg, G.V. & Böttger, E.C. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. *Eur Respir J* 45, 554-7 (2015).

(c) Böttger, unpublished data.

9. Werngren, unpublished data.

3.B.1 BDQ MIC data on LJ

3.B.1.1 BDQ MICs for pWT isolates on LJ

No studies presented MIC distributions for pWT isolates on LJ.

3.B.1.2 BDQ MICs for mutated isolates on LJ

No studies were found.

3.B.1.3 Conclusion for BDQ CC for LJ

Given that no MIC data were identified for LJ, a CC could not be defined.

3.B.2 BDQ MIC data on 7H10

3.B.2.1 BDQ MICs for pWT isolates on 7H10

Three studies reported BDQ MIC data for the pWT population on 7H10 (Table 52):

1. Kaniga *et al.* (Study 1) conducted a study to determine the QC range for H37Rv ATCC 27294. This strain was tested 30 times in eight different centres. The results from laboratory 5 were excluded from the analysis by Kaniga *et al.*, as the MICs from this laboratory were found to have unusually high MICs for one medium lot, which resulted in a bimodal MIC distribution. Excluding these data left 213 replicates of H37Rv, with MICs that ranged from ≤0.008-0.25 mg/L with a mode at 0.06 mg/L. Based on these findings, 0.12 mg/L was defined as the upper end of the H37Rv QC range, which included 211 of the MICs (99% (95% CI, 97-100%)).

2. Ismail *et al.* (Study 2) conducted a large, retrospective study of South African MTBC clinical isolates. WGS was conducted on all isolates. The MIC range for the 37 replicates of H37Rv ATCC 27294 tested in this study was 0.03-0.06 mg/L (with a mode at 0.06 mg/L), which was in agreement with the aforementioned QC range from Study 1, in which this laboratory participated. In contrast, the 371 gWT clinical isolates that had not been exposed to BDQ showed an unusually wide MIC range, with MICs spanning eight dilutions (0.015 to >2 mg/L).[13] Notably, the mode of 0.25 mg/L of this distribution was two dilutions higher than the mode of the QC range from Study 1. When this same set of clinical isolates was tested in MGIT (as part of Study 12 in Section 3.B.4.1), the resulting mode of the pWT MIC distribution was within one dilution of the mode of the H37Rv MIC distribution in MGIT. Moreover, the MIC distribution for these clinical isolates (as well as the H37Rv MIC distribution from Study 12) was comparable

13 European Committee for Antimicrobial Susceptibility Testing. Standard Operating Procedure. MIC distributions and the setting of epidemiological cutoff (ECOFF) values. 17 November 2017.

to the H37Rv and clinical MIC data in MGIT from another laboratory (Study 11). Together, these observations suggest a methodological problem with Ismail *et al.* (Study 2), which was therefore excluded from further analysis in this report. This observation underscored the need for careful standardisation of BDQ testing, which is more prone to variability than other drugs given that a variety of factors can affect MIC results (e.g. the type of plastic used, or protein (albumin) binding).[14]

3. Diacon *et al.* (Study 3) presented MICs for 44 clinical isolates from an early BDQ bactericidal activity trial. 33 MICs were truncated at the lower end of the distribution, which precluded an assessment of the mode of the distribution.

Table 52. BDQ MICs for pWT isolates on 7H10.

							BDQ MIC (mg/L)									
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	3.2
1) Kaniga 2016	1	lab strain	1	30	H37Rv ATCC 27294				13	8	9					
	2		1	33	H37Rv ATCC 27294		1	5	**22**	5						
	3		1	30	H37Rv ATCC 27294				10	**12**	8					
	4		1	30	H37Rv ATCC 27294					**30**						
	5		1	30	H37Rv ATCC 27294		4	**9**	6	1	1	**9**				
	6		1	30	H37Rv ATCC 27294		6	10	**14**							
	7		1	30	H37Rv ATCC 27294			8	**12**	9		1				
	8		1	30	H37Rv ATCC 27294				8	**17**	4	1				
1) Kaniga 2016	1-4 & 6-8	sum of dataset 1, excluding lab 5	1	213			7	23	79	**81**	21	2				
2) Ismail	1a		1	37	H37Rv ATCC 27294				6	**31**						
	1a		371	371	no BDQ exposure (different degrees of R)	gWT		2	8	60	91	**140**	60	6	2	2
	1a	clinical	9	9	BDQ exposed (mostly XDR)	gWT				1	1	5	2			
	1a		2	2	XDR patients failing on BDQ	gWT							2			
3) Diacon 2012	9		1	1	H37Rv ATCC 27294				1							
	9	clinical	44	44					33	11						

The purple line denotes the current EUCAST CB for BDQ testing (0.25 mg/L). Notable limitations: The laboratory in which Study 2 was conducted also participated in Study 1 (because this was a blinded study, it was unclear what the corresponding laboratory number was). Study 2 had an unusually wide pWT MIC distribution.

3.B.2.2 BDQ MICs for mutated isolates on 7H10

The data from Ismail *et al.* (Study 2), which featured mutated clinical isolates, were excluded (Section 3.B.2.1). However, these data can be found in the supplementary MIC file.

3.B.2.3 Conclusion for BDQ CC for 7H10

Because data from Ismail *et al.* (Study 2) were excluded for methodological reasons, only H37Rv QC data from and a truncated pWT MIC distribution were available for consideration. These data were insufficient to propose a CC.

3.B.3 BDQ MIC data on 7H11

3.B.3.1 BDQ MICs for pWT isolates on 7H11

Six studies reported BDQ MIC data for the pWT population on 7H11 (Table 53). Five of these studies either reported MICs for more than 10 pWT isolates or did extensive repeat MIC resting:

1. Kaniga *et al.* (Study 4) conducted a study to determine the QC range for H37Rv ATCC 27294. This strain was tested 30-32 times in eight different centres. The pooled MIC distribution of the total 242 replicates ranged from ≤0.008 to 0.25 mg/L (with a mode at 0.06 mg/L). Notably, the nine MICs at the higher end of the distribution were all from laboratory 5, which was excluded by Kaniga *et al.* from the evaluation of MIC data for 7H10 (Section 3.B.2.1). Based on these findings, 0.12 mg/L was defined as the upper end of the QC range, which included 233 of the total MICs (96% (95% CI, 93-98%)).

2. Diacon *et al.*, Pym *et al.* and Villellas *et al.* (Study 5) presented MIC data for 325 gWT baseline clinical isolates from trials C208 and C209. The corresponding pWT MIC distribution

14 Lounis, N., Vranckx, L., Gevers, T., Kaniga, K. & Andries, K. *In vitro* culture conditions affecting minimal inhibitory concentration of bedaquiline against *M. tuberculosis*. *Med Mal Infect* 46, 220-5 (2016).

ranged from ≤0.008 to 0.25 mg/L (with a mode at 0.06 mg/L). This study also included data for four post-baseline isolates from trial C209 that were either gWT (n=3) or for which no genotypic information was available (n=1). The MICs of these isolates were 0.03-0.06 mg/L. The relationship between baseline BDQ MIC and culture conversion rates can be found in Supplementary Table 2.

3. Torrea *et al.* (Study 6) presented additional data from the laboratory that did the testing for Study 5. The pWT MIC distribution was ≤0.008-0.25 mg/L (with a mode at 0.06 mg/L) for 77 clinical isolates.

4. Andries *et al.* (Study 7) reported a pWT MIC distribution of ≤0.008-0.12 mg/L for 22 clinical isolates. The distribution was bimodal with one mode at 0.015 and another at 0.06 mg/L.

5. Zimenkov *et al.* (Study 8) reported a pWT MIC distribution of ≤0.03-0.12 mg/L for 21 clinical isolates. However, the MIC distribution of 15 isolates was truncated at the lower end of the distribution, which precluded an assessment of its shape. The MIC of H37Rv ATCC 25618 was also truncated at ≤0.03 mg/L.

Table 53. BDQ MICs for pWT isolates on 7H11.

							BDQ MIC (mg/L)							
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1
4) Kaniga 2016	1	lab strain	1	30	H37Rv ATCC 27294			1	**12**	11	6			
	2		1	32	H37Rv ATCC 27294		1	3	**25**	3				
	3		1	30	H37Rv ATCC 27294				**23**	7				
	4		1	30	H37Rv ATCC 27294					**30**				
	5		1	30	H37Rv ATCC 27294				3	**13**	5	9		
	6		1	30	H37Rv ATCC 27294			**13**	8	9				
	7		1	30	H37Rv ATCC 27294			10	**12**	8				
	8		1	30	H37Rv ATCC 27294				5	**24**	1			
4) Kaniga 2016	1-8	sum of dataset 5	1	242	H37Rv ATCC 27294		1	27	88	**105**	12	9		
5) Diacon 2014a &	10	clinical	325	325	baseline isolates from trial 208 & 209	gWT	4	35	77	**157**	49	3		
2014b, Pym 2016 &	10		1	1	post-baseline isolates from trial 209				**1**					
Villellas 2016	10		3	3	post-baseline isolates from trial 209	gWT			1	**2**				
6) Torrea 2015	10	clinical	77	77	different levels of R		2	3	13	**42**	16	1		
7) Andries 2005	11	clinical	1	2	H37Rv				1	1				
	11		22	22			3	**7**	2	**8**	2			
8) Veziris 2017	12	clinical	1	1	H37Rv				**1**					
	12		1	1	MDR	gWT							1	
	12		1	1	MDR	gWT parent		1						
9) Zimenkov 2017	13	clinical	1	1	H37Rv ATCC 25618				1					
	13		21	21	mostly XDR	gWT			15	4	2			

The purple line denotes the current EUCAST CB for BDQ testing (0.25 mg/L). Notable limitation: Studies 5 and 6 were conducted in the same laboratory.

3.B.3.2 BDQ MICs for mutated isolates on 7H11

In vitro and clinical isolates

Torrea *et al.* (Study 6) reported MICs of 1-2 mg/L for 15 *in vitro mmpR* mutants. One mutant with an unknown resistance mechanism had an MIC of 1 mg/L, whereas an *in vitro atpE* mutant had an MIC >2 mg/L (Table 54).

Four studies featured MIC data for clinical *mmpR* mutants (Table 54):

1. Torrea *et al.* (Study 6) reported one naturally resistant MDR *mmpR* mutant with an MIC of 0.5 mg/L.

2. Diacon *et al.*, Pym *et al.* and Villellas *et al.* (Study 5) reported MICs for *mmpR* mutants from two groups of isolates:

a. Eight baseline isolates from trials 208 and 209 had *mmpR* LOF mutations. Two of the isolates had MICs ≤0.008 mg/L, one had an MIC of 0.06 mg/L and the remaining five had MICs of at least 0.25 mg/L. 14 additional baseline isolates had other *mmpR* mutations, with MICs of 0.015-0.5 mg/L.

3. Seven *mmpR* LOF mutants from post-baseline isolates from trial C209 had MICs of 0.12 to >0.5 mg/L, whereas eight other *mmpR* mutants had MICs of 0.06 to >0.5 mg/L

(Supplementary Table 3 shows the MICs of all post-baseline isolate from this trial compared to their respective baseline isolates). Veziris *et al.* (Study 8) described two isolates with MICs of 0.25 and 0.5 mg/L that had different *mmpR* LOF mutations. Moreover, a *mmpR* mutant that was isolated from a patient failing therapy (with a regimen that included BDQ) had an MIC of 0.25 mg/L compared with ≤0.015 mg/L for the gWT parental isolate at baseline.

4. Zimenkov *et al.* (Study 9) reported 22 *mmpR* mutants with MICs of 0.06-0.25 mg/L. Moreover, they reported that one isolate with a baseline *mmpR* L142R mutation acquired an *atpE* A63V mutation during BDQ treatment, which resulted in a BDQ MIC increase from 0.25 mg/L to 1 mg/L (Supplementary Table 4 shows the isolates that acquired either *mmpR* or *atpE* mutations during the course of treatment in this study). Finally, the acquisition of an *atpE* D28N mutation resulted in an MIC increase from 0.03 mg/L to 0.12 mg/L. Notably, different mutations in both of these *atpE* codons had been previously described in BDQ-resistant *in vitro* mutants.[15, 16] Even if 0.12 mg/L was used as the concentration to define resistance, 50% (95% CI, 29-71%) of these 24 mutants tested, including the *atpE* D28N mutant, would be deemed BDQ-susceptible despite the trend towards higher MICs for isolates that developed mutations during treatment. In light of the severe truncation of the pWT MIC distribution in this study, including the MIC for H37Rv, it was not possible to assess whether this was due to a methodological difference in MIC testing. As this is the first study to feature clinical *atpE* mutants, retesting of these isolates using standardised protocols and multiple media would be valuable.

Table 54. BDQ MICs for in vitro and clinical atpE and mmpR mutants on 7H11.

						BDQ MIC (mg/L)										
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	*0.008*	*0.015*	*0.03*	*0.06*	*0.12*	*0.25*	*0.5*	*0.8*	*1*	*2*	*3.2*
6) Torrea 2015	10		15		*mmpR* mutants									13	2	
	10	*in vitro* mutants	1		unknown mechanism									1		
	10		1		*atpE* mutant											1
	10	clinical	1	MDR	natural *mmpR* LOF mutant							1				
5) Diacon 2014a & 2014b, Pym 2016 & Villellas 2016	10	clinical	8	baseline isolates from trial 208 & 209	*mmpR* LOF mut	2			1		2	1	2			
	10		14	baseline isolates from trial 208 & 209	*mmpR* other mutations		2	1	2	4		5				
	10		7	post-baseline isolates from trial 209	*mmpR* LOF mut					1	1	4	1			
	10		8	post-baseline isolates from trial 209	*mmpR* other mutations				1	2		1	4			
8) Veziris 2017	12	clinical	2	MDR/XDR	natural *mmpR* LOF mutant (2 different mut.)						1	1				
	12		1	MDR	gWT parent		1									
	12		1	MDR	*mmpR* mutant from patient failing therapy that included BDQ						1					
9) Zimenkov 2017	13	clinical	22	mostly XDR	*mmpR* mutants				2	9	11					
	13		1	mostly XDR	*mmpR* & *atpE* A63V									1		
	13		1	mostly XDR	*atpE* D28N					1						

The purple line denotes the current EUCAST CB for BDQ testing (0.25 mg/L). Notable limitation: Studies 5 and 6 were conducted in the same laboratory.

3.B.3.3 Conclusion for BDQ CC for 7H11

An interim CC of **0.25 mg/L** was adopted based on the H37Rv QC and pWT MICs, even though most clinical MIC data were from a single laboratory. However, this CC did not necessarily allow for the adequate differentiation of clinical *mmpR* mutants from the pWT population. Some *mmpR* mutants might have had genuinely low MICs if the mutations in question were genetic polymorphisms that did not affect the action of the repressor. Other *mmpR* mutations may only have minor functional consequences and may therefore only result in slight MIC increases, leading to a gNWT MIC distribution that overlaps with the pWT MIC distribution.

15 Huitric, E. *et al.* Rates and mechanisms of resistance development in *Mycobacterium tuberculosis* to a novel diarylquinoline ATP synthase inhibitor. *Antimicrob Agents Chemother* 54, 1022-8 (2010).
16 Preiss, L. *et al.* Structure of the mycobacterial ATP synthase Fo rotor ring in complex with the anti-TB drug bedaquiline. *Sci Adv* 1, e1500106 (2015)

3.B.4 BDQ MIC data in MGIT

3.B.4.1 BDQ MICs for pWT isolates in MGIT

Three studies presented BDQ MIC data for the pWT population with MGIT (Table 55):

1. Bloemberg *et al.*, Keller *et al.* and Somoskovi *et al.* (Study 10) used a different dilution series than the other studies and used crushed BDQ tablets rather than pure compound for MIC testing. The pWT MIC distribution for the total 22 clinical isolates tested was 0.2-1.6 mg/L (with a mode at 0.4 mg/L).

2. Torrea *et al.* (Study 11) reported an MIC distribution of 0.12-1 mg/L (with a mode at 0.25 mg/L) for 37 replicates of H37Rv ATCC 27294. 72 clinical isolates had a pWT MIC distribution of ≤0.03-1 mg/L (with a mode at 0.25 mg/L). Moreover, two additional clinical isolates were retested for a total of 17 times, which resulted in a MIC distribution of 0.12-1 mg/L (with two modes: at 0.25 mg/L and 1 mg/L).

3. Ismail *et al.* (Study 12) retested the same collection that had been tested on 7H10 (the data on that medium had to be excluded for methodological reasons (Section 3.B.2.1)). 31 replicates of H37Rv ATCC 27294 had an MIC distribution of ≤0.12-1 mg/L (with a mode at 0.25 mg/L). Moreover, 382 gWT clinical isolates (mostly from patients without prior exposure to BDQ) were tested. A pWT MIC distribution of approximately ≤0.12-2 mg/L (with a mode at 0.5 mg/L) was reported. The MICs of a proportion of these isolates were repeated (Supplementary Table 5).

Table 55. BDQ MICs for pWT isolates in MGIT.

						BDQ MIC (mg/L)												
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	*0.03*	*0.06*	*0.1*	*0.12*	*0.2*	*0.25*	*0.4*	*0.5*	*0.8*	*1*	*1.6*	*2*	*4*
10) Bloemberg 2015, Keller 2015 & Somoskovi 2015	clinical	1	1	H37Rv ATCC 27294								**1**						
		10	10	pan-S						1		**5**		4				
		11	11	MDR								**5**		4		2		
		1	1	XDR	gWT									1				
11) Torrea 2015	clinical	1	37	H37Rv ATCC 27294					7		**19**		10		1			
		72	72	different levels of R		3	1		13		**33**		17		5			
		2	17	different levels of R					1		**5**		3		**8**			
12) Ismail	clinical	1	31	H37Rv ATCC 27294					8		**13**		8		2			
		371	371	no BDQ exposure (different degrees of R)	gWT				52		88		**183**		45		2	1
		9	9	BDQ exposed (mostly XDR)	gWT						2		4		1		2	
		2	2	XDR patients failing on BDQ	gWT												2	

The purple line denotes the current EUCAST CB for BDQ testing (0.25 mg/L). Notable limitation: Study 10 did not use pure BDQ for MIC testing.

3.B.4.2 BDQ MICs for mutated isolates in MGIT

In vitro and clinical isolates

Torrea *et al.* (Study 11) reported MICs of ≥2 mg/L for 14 *in vitro mmpR* mutants (Table 56). One additional *in vitro mmpR* mutant was tested nine times, which resulted in MICs of >2 mg/L in all cases. In the same study, one mutant with an unknown resistance mechanism also had an MIC of >2 mg/L, as did the nine replicates of an *in vitro atpE* mutant.

Three studies reported MIC data for clinical *mmpR* or *pepQ* mutants (Table 56).

1. Torrea *et al.* (Study 11) reported an MIC of >2 mg/L for a naturally resistant *mmpR* clinical mutant.

2. Bloemberg *et al.*, Keller *et al.* and Somoskovi *et al.* (Study 10) reported an MIC of 6.4 mg/L for two replicates of an *mmpR* mutant that developed in response to treatment with CFZ, compared with 0.8 mg/L for the gWT parental isolate (this mutant was also cross-resistant to CFZ (Section 3.A.4.2)). It should be noted that a major limitation of this study was that crushed, rather than pure, BDQ compound was used for testing.Ismail *et al.* (Study 12) observed BDQ MICs of 2-8 mg/L for nine *mmpR* mutants, which included six isolates from XDR patients who were failing BDQ therapy. Of the remaining three *mmpR* mutants, only one was from a patient with a prior history of BDQ treatment (repeat testing of these mutants yielded MICs of 1-2 mg/L (Supplementary Table 5)). Four isolates harboured three different *pepQ* mutations with MICs of 0.5-1 mg/L.

Table 56. BDQ MICs for in vitro and clinical atpE and mmpR mutants in MGIT.

						BDQ MIC (mg/L)										
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	0.2	0.25	0.4	0.5	0.8	1	1.6	2	3.2	4	6.4	8
11 Torrea 2015	*in vitro* mutants	14	14		*mmpR* mutants								1	13			
		1	9		*mmpR* mutant									9			
		1	1		unknown mechanism									1			
		1	9		*atpE* mutant									9			
	clinical	1	1	MDR	natural *mmpR* LOF mutant									1			
10) Bloemberg 2015, Keller 2015 &	clinical	1	1	XDR	gWT parent					1							
		1	2	XDR	*mmpR* V1A											2	
12) Ismail	clinical	2	2	no BDQ exposure (different degrees of R)	*mmpR* mutants (2 different mut.)								1		1		
		1	1	BDQ exposed (mostly XDR)	*mmpR* LOF mut										1		
		4	4	XDR patients failing on BDQ	*mmpR* LOF mut (3 different mut.)								2		2		
		2	2	XDR patients failing on BDQ	*mmpR* mutants (1 mut.)										1		1
		4	4	no BDQ exposure (different degrees of R)	*pepQ* mutants (3 different mut.)				2		2						

The purple line denotes the current EUCAST CB for BDQ testing (0.25 mg/L). Notable limitation: Study 10 did not use pure BDQ for MIC testing.

3.B.4.3 Conclusion for BDQ CC for MGIT

Based on the pWT and H37Rv MIC data from Studies 11 and 12, an interim CC of **1 mg/L** for MGIT was adopted. The findings of Study 10 supported this value, despite having used crushed BDQ and a different dilution series. All 16 *mmpR* or *atpE in vitro* mutants tested (100% (95% CI, 79-100%)) and all 10 clinical *mmpR* mutants tested (100% (95% CI, 69-100%)) would be interpreted as BDQ-resistant at this concentration. However, even in MGIT there appeared to be some overlap between the *mmpR* MIC distributions and the pWT population, as demonstrated by repeat MIC testing (i.e. the three *mmpR* mutants from Ismail *et al.* (Study 11) with initial BDQ MICs of 2-4 mg/L had MICs of 1-2 mg/L upon retesting (Supplementary Table 5)).

3.B.5 References for BDQ MIC studies

1. Kaniga, K. *et al.* A multilaboratory, multicountry study to determine bedaquiline MIC quality control ranges for phenotypic drug susceptibility testing. *J Clin Microbiol* 54, 2956-2962 (2016).

2. Ismail, unpublished data.

3. Diacon, A.H. *et al.* 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet* 380, 986-93 (2012).

4. Kaniga, K. *et al.* A multilaboratory, multicountry study to determine bedaquiline MIC quality control ranges for phenotypic drug susceptibility testing. *J Clin Microbiol* 54, 2956-2962 (2016).

5. (a) Diacon, A.H. *et al.* Multidrug-resistant tuberculosis and culture conversion with bedaquiline. *N Engl J Med* 371, 723-32 (2014).

(b) Diacon, A.H., Lounis, N. & Dannemann, B. Multidrug-resistant tuberculosis and bedaquiline. *N Engl J Med* 371, 2436 (2014).

(c) Pym, A.S. *et al.* Bedaquiline in the treatment of multidrug- and extensively drug-resistant tuberculosis. *Eur Respir J* 47, 564-74 (2016).

(d) Villellas, C. *et al.* Unexpected high prevalence of resistance-associated Rv0678 variants in MDR-TB patients without documented prior use of clofazimine or bedaquiline. *J Antimicrob Chemother* 72, 684-690 (2017).

6. Torrea, G. *et al.* Bedaquiline susceptibility testing of *Mycobacterium tuberculosis* in an automated liquid culture system. *J Antimicrob Chemother* 70, 2300-5 (2015).

7. Andries, K. *et al.* A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis. Science* 307, 223-7 (2005).

8. Veziris, N. *et al.* Rapid emergence of *Mycobacterium tuberculosis* bedaquiline resistance: lessons to avoid repeating past errors. *Eur Respir J* 49, 1601719 (2017).

9. Zimenkov, D.V. *et al.* Examination of bedaquiline- and linezolid-resistant *Mycobacterium tuberculosis* isolates from the Moscow region. *J Antimicrob Chemother* 72, 1901-1906 (2017)

10. (a) Bloemberg, G.V. *et al.* Acquired resistance to bedaquiline and delamanid in therapy for tuberculosis. *N Engl J Med* 373, 1986-8 (2015).

(b) Keller, P.M. *et al.* Determination of MIC distribution and epidemiological cutoff values for

bedaquiline and delamanid in *Mycobacterium tuberculosis* using the MGIT 960 System equipped with TB eXiST. *Antimicrob Agents Chemother* 59, 4352-5 (2015).

(c) Somoskovi, A., Bruderer, V., Hömke, R., Bloemberg, G.V. & Böttger, E.C. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. *Eur Respir J* 45, 554-7 (2015).

11. Torrea, G. *et al.* Bedaquiline susceptibility testing of *Mycobacterium tuberculosis* in an automated liquid culture system. *J Antimicrob Chemother* 70, 2300-5 (2015).

12. Ismail, unpublished data.

4. Cycloserine and terizidone

4.0 DCS and TRD MIC data stratification and current breakpoints

No MIC data were identified for TRD. DCS MIC data were stratified based on *ald* (Rv2780) and *alr* (*Rv3423c*) mutations (a detailed discussion of the known resistance mechanisms for this drug can be found in the supplement). The start codon of *alr* has been wrongly annotated in the H37Rv reference genome (GenBank accession number AL123456.3), and therefore the experimentally confirmed start codon was used to report mutations in this gene.[17]

The only CC for DCS was set by WHO (Table 57).[18] No CC currently exists for TRD DST.

Table 57. Overview of current DCS and TRD CCs.

LJ		7H10		7H11		MGIT	
WHO	CLSI	WHO	CLSI	WHO	CLSI	WHO	CLSI
30.0	–	–		–		–	

Red CCs (in mg/L) were set by only WHO.

4.1 DCS MIC data on LJ

4.1.1 DCS MICs for pWT isolates on LJ

Nakatani *et al.* (Study 1) only reported MICs for H37Rv tested in two laboratories and thus provided little insight into the pWT MIC distribution (Table 58).

Table 58. DCS MICs for pWT and mutated isolates on LJ.

						DCS MIC (mg/L)									
Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic results	3.75	7.5	10	15	20	30	40	60	120	240
1) Nakatani 2017	1	clinical	1	H37Rv ATCC 27294			1								
	1		1	MDR	gWT parent				1						
	1		1	MDR	*alr* c-8t								1		
	2		1	H37Rv ATCC 27294				1							
	2		1	XDR	*alr* M319T							1			
	2		1	XDR	*alr* Y364D							1			
	2		1	XDR	*ald* LOF & *alr* R373L							1			

The red line denotes the current WHO CC for DCS on LJ (30 mg/L).

4.1.2 DCS MICs for mutated isolates on LJ

Clinical isolates

Nakatani *et al.* (Study 1) demonstrated that the acquisition of an *alr* mutation (C to T at position -8 relative to the start of the gene) during MDR treatment correlated with a DCS MIC increase from 15 to 60 mg/L (Table 58). Three additional *alr* mutations, one of which coincided with an *ald* mutation, also correlated with MICs above the current CC. Nakatani *et al.* provided additional evidence by molecular modelling and direct measurements of enzymatic activity that these *alr* mutations are likely responsible for DCS resistance.

17 Strych, U., Penland, R.L., Jimenez, M., Krause, K.L. & Benedik, M.J. Characterization of the alanine racemases from two mycobacteria. *FEMS Microbiol Lett* 196, 93-8 (2001).
18 World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1 (accessed 13.8.2015).

4.1.3 Conclusion for DCS CC for LJ

As the data quantity was insufficient to propose even a tentative CC, the current CC of 30 mg/L was withdrawn.

4.2 DCS MIC data on 7H10

4.2.1 DCS MICs for pWT isolates on 7H10

Two studies were identified that reported DCS MIC data for the pWT population on 7H10 (Table 59). Schön *et al.* (Study 2) tested 110 clinical isolates that had a pWT MIC distribution of 8-32 mg/L (with a mode at 32 mg/L). Pholwat *et al.* (Study 3) reported a pWT MIC distribution of 3.75-15 mg/L (with a mode at 15 mg/L) for 21 clinical isolates.

Table 59. DCS MICs for pWT isolates on 7H10.

					DCS MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**1.87**	**3.75**	**4**	**7.5**	**8**	**15**	**16**	**30**	**32**
2) Schön 2011		1	4	H37Rv ATCC 27294							**4**		
	clinical	110	110	different levels of R					10		41		**59**
3) Pholwat 2011,		1	1	H37Rv ATCC 27294						**1**			
2012 & 2015	clinical	21	21	different levels of R		1		9		**11**			

4.2.2 DCS MICs for mutated isolates on 7H10

No studies presenting MICs for mutated isolates were identified.

4.2.3 Conclusion for DCS CC for 7H10

Only two studies were identified that reported DCS MICs for 7H10. These data were deemed insufficient to propose a CC.

4.3 DCS MIC data on 7H11

4.3.1 DCS MICs for pWT isolates on 7H11

Only one study was identified that reported DCS MIC data for the pWT population on 7H11 (Table 60). Fattorini *et al.* (Study 4) tested 46 clinical isolates, which were enriched for resistance to other drugs, and found an MIC distribution of 7.5-60 mg/L (with a mode at 15 mg/L).

Table 60. DCS MICs for pWT isolates on 7H11.

				DCS MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**3.75**	**7.5**	**15**	**30**	**60**	**120**
4) Fattorini 1999		1	H37Rv ATCC 27294		**1**				
	clinical	46	R to at least 2 first-line drugs		2	**22**	18	4	

4.3.2 DCS MICs for mutated isolates on 7H11

No studies presenting MIC distributions for mutated isolates were identified.

4.3.3 Conclusion for DCS CC for 7H11

A single study was identified, which was insufficient to set a CC.

4.4 DCS MIC data in MGIT

4.4.1 DCS MICs for pWT isolates in MGIT

Only one study was identified that used MGIT to determine DCS MICs (Table 61). Nakatani *et al.* (Study 5) tested four closely-related clinical isolates, which all had a MIC of 16 mg/L compared to an MIC of 4 mg/L for H37Rv ATCC 27294.

Table 61. DCS MICs for pWT and mutated isolates in MGIT.

					DCS MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**2**	**4**	**8**	**16**	**32**	**64**
5) Nakatani 2017	clinical	1	H37Rv ATCC 27294			**1**				
		4	different levels of R	gWT				**4**		
		3	MDR & XDR	*alr* M319T						3

4.4.2 DCS MICs for mutated isolates in MGIT

Clinical isolates

The *alr* mutation M319T, which has been predicted to result in DCS resistance through molecular modelling,[19] was shown to correlate with a DCS MIC of 64 mg/L in MGIT compared to an MIC of 16 mg/L for closely-related *alr* wild type control isolates (Table 61).

4.4.3 Conclusion for DCS CC for MGIT

The data from the single study identified were insufficient to set a CC.

4.5 References for DCS MIC studies

1. Nakatani, Y. *et al.* Role of alanine racemase mutations in *Mycobacterium tuberculosis* D-cycloserine resistance. *Antimicrob Agents Chemother* 61, e01575-17 (2017).

2. Schön, T. *et al.* Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis. Int J Tuberc Lung Dis* 15, 502-9 (2011).

3. (a) Pholwat, S., Heysell, S., Stroup, S., Foongladda, S. & Houpt, E. Rapid first- and second-line drug susceptibility assay for *Mycobacterium tuberculosis* isolates by use of quantitative PCR. *J Clin Microbiol* 49, 69-75 (2011).

(b) Pholwat, S., Ehdaie, B., Foongladda, S., Kelly, K. & Houpt, E. Real-time PCR using mycobacteriophage DNA for rapid phenotypic drug susceptibility results for *Mycobacterium tuberculosis. J Clin Microbiol* 50, 754-61 (2012).

(c) Pholwat, S. *et al.* Integrated microfluidic card with TaqMan probes and high-resolution melt analysis to detect tuberculosis drug resistance mutations across 10 genes. *MBio* 6, e02273 (2015).

4. Fattorini, L. *et al.* Activity of 16 antimicrobial agents against drug-resistant strains of *Mycobacterium tuberculosis. Microb Drug Resist* 5, 265-70 (1999).

5. Nakatani, Y. *et al.* Role of alanine racemase mutations in *Mycobacterium tuberculosis* D-cycloserine resistance. *Antimicrob Agents Chemother* 61, e01575-17 (2017).

19 Köser, C.U. *et al.* Whole-genome sequencing for rapid susceptibility testing of *M. tuberculosis*. *N Engl J Med* 369, 290-2 (2013).

5. Linezolid

5.0 LZD MIC data stratification and current breakpoints

LZD MIC data were stratified based on mutations in the *rrl* (*MTB000020*) and *rplC* (*Rv0701*) genes (a detailed discussion of the known resistance mechanisms for this drug can be found in the supplement). In presenting the nucleotide changes for *rrl* mutants, two numbers were given: the first number represents the nucleotide position in *M. tuberculosis*, whereas the second number represents the corresponding nucleotide position in *Escherichia coli*. The latter numbering system is more commonly used to report mutations in this gene.

To date, CLSI and WHO have only set a CC for LZD DST in MGIT (Table 62).[20, 21]

Table 62. Overview of current LZD CCs.

LJ		7H10		7H11		MGIT	
WHO	CLSI	WHO	CLSI	WHO	CLSI	WHO	CLSI
–		–		–		1.0	

Green CCs (in mg/L) were set by both WHO and CLSI.

5.1 LZD MIC data on LJ

5.1.1 LZD MICs for pWT isolates on LJ

No studies presenting MIC distributions for pWT isolates on LJ were identified.

5.1.2 LZD MICs for mutated isolates on LJ

No studies with MIC distributions for mutated isolates on LJ were found.

5.1.3 Conclusion for LZD CC for LJ

Given that no MIC data were identified for LJ, a CC could not be defined.

5.2 LZD MIC data on 7H10

5.2.1 LZD MICs for pWT isolates on 7H10

10 studies were identified that reported LZD MIC data for the pWT population on 7H10 (Table 63). The distribution in the study by Wang *et al.* (Study 1) was bimodal and consequently had to be excluded based on EUCAST rules for aggregating MIC data.[22] The remaining nine studies featured more than 650 MICs for pWT isolates with modes at either 0.25 or 0.5 mg/L. These data suggested a CC of 1 mg/L for DST on 7H10.

20 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).
21 World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1 (accessed 13.8.2015).
22 European Committee for Antimicrobial Susceptibility Testing. Standard Operating Procedure. MIC distributions and the setting of epidemiological cutoff (ECOFF) values. 17 November 2017.

Table 63. LZD MICs for pWT isolates on 7H10.

					LZD MIC (mg/L)							
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
1) Wang 2007	clinical	420	420	different levels of R	7	28	**125**	39	**204**	16	1	
2) Weiss 2015	clinical	1	20	H37Rv		1	**14**	5				
		130	130	non-MDR		4	**121**	5				
		18	18	MDR			**10**	8				
3) Schön 2011	clinical	1	4	H37Rv ATCC 27294			**4**					
		110	110	mostly pan-S		2	**91**	17				
4) Ahmed 2013	clinical	1	1	H37Rv ATCC 27294			**1**					
		43	43	pre-XDR		5	**35**	3				
		59	59	XDR		6	**50**	2	1			
5) McGee 2009	clinical	1	1	H37Rv ATCC 27294					**1**			
		19	19					**10**	9			
6) Koh 2009	clinical	1	1	H37Rv ATCC 25618				**1**				
		10	10	MDR & XDR			3	**7**				
7) Alcalá 2003	clinical	1	1	H37Rv ATCC 27294			**1**					
		105	105	mostly pan-S		1	4	**87**	13			
		12	12	MDR			5	**7**				
8) Yang 2012	clinical	1	1	H37Rv ATCC 27294				**1**				
		15	15	pan-S		1	**12**	2				
		8	8	INH-R		1	**7**					
		45	45	MDR		5	**40**					
		16	16	XDR		3	**13**					
9) Pholwat 2011, 2012 & 2015	clinical	1	1	H37Rv ATCC 27294					**1**			
		37	37	different levels of R		1	**26**	10				
10) van Ingen 2010	clinical	1	1	H37Rv ATCC 27294			**1**					
		28	28	MDR		13	**14**	1				

Notable limitation: The distribution in Study 1 was bimodal.

5.2.2 LZD MICs for mutated isolates on 7H10

No studies presenting MIC distributions for gNWT strains on 7H10 were identified.

5.2.3 Conclusion for LZD CC for 7H10

A CC of **1 mg/L** was adopted for DST on 7H10.

5.3 LZD MIC data on 7H11

5.3.1 LZD MICs for pWT isolates on 7H11

Three studies were identified that reported LZD MIC data for the pWT population on 7H11 (Table 64). These studies featured more than 300 isolates, and only two MICs were truncated at the lower end of the pWT MIC distributions, which had modes of either 0.25 or 0.5 mg/L. These data suggest a CC of 1 mg/L for DST on 7H11.

Table 64. LZD MICs for pWT isolates on 7H11.

				LZD MIC (mg/L)									
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
11) Rodríguez 2002	clinical	243	mostly pan-S	2	4	100	**125**	9					3
12) Kazemian 2015	clinical	1	H37Rv			**1**							
		40	MDR & XDR			**28**	8						4
13) Rey-Jurado 2013a, Rey-Jurado 2013b & López-Gavín 2016	clinical	1	H37Rv				**1**						
		11	pan-S			1	**9**	1					
		9	MDR				**7**	2					

5.3.2 LZD MICs for mutated isolates on 7H11

No studies presenting MIC distributions for gNWT strains on 7H11 were identified.

5.3.3 Conclusion for LZD CC for 7H11

A CC of **1 mg/L** for DST on 7H11 was adopted.

5.4 LZD MIC data in MGIT

5.4.1 LZD MICs for pWT isolates in MGIT

12 studies were identified that reported LZD MIC data for the pWT population in MGIT (Table 65). Their distributions, including in Rüsch-Gerdes *et al.* (Study 23), which has been cited by the current CLSI guidelines to support a CC of 1 mg/L for MGIT, were often truncated at the lower or upper ends.[23] Therefore little information regarding the shape of the distributions could be obtained. Where the modes could be defined, they varied between 0.25 to 1 mg/L, suggesting more inter-laboratory variation than on 7H10 and 7H11 (Sections 5.2.1 and 5.3.1).

Table 65. LZD MICs for pWT isolates in MGIT.

							LZD MIC (mg/L)							
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.12	0.25	0.5	1	2	4	8	16
14) Yip 2013	14		1	1	H37Ra ATCC 25177				**1**					
	14		7	7	pan-S				3	**4**				
	14	clinical	31	31	MDR			3	9	**17**	2			
	14		9	9	XDR				2	**5**	2			
15) Richter 2007 & Beckert 2012	15		1	1	H37Rv ATCC 27294					**1**				
	15	clinical	1	1	MDR				**1**					
	15		4	4	MDR	gWT parental strain			**3**	1				
16) Dheda 2017	16	clinical	149	149	XDR		21	**116**	11	1				
17) Ismail	17	clinical	68	68	different levels of R			5	**40**	8	2	5	6	2
18) van Ingen 2010	10		1	1	H37Rv ATCC 27294					**1**				
	10	clinical	28	28	MDR			14	**14**					
19) Alffenaar 2011	10	clinical	23	23	different levels of R			6	**8**	**8**	1			
20) Werngren	18	clinical	26	26	different levels of R			7	**17**	2				
21) Sharma 2011	19-22	clinical	36	144	different levels of R				67	62	4	11		
22) Mirza 2015	23		1	1	H37Rv ATCC 27294				1					
	23	clinical	100	100	MDR				80	16		4		
23) Rüsch-Gerdes 2006	15, 24-25	clinical	10	30	H37Rv ATCC 27294 & pan-S				20	9	1			
	15, 24-25		21	63	different levels of R				55	6	2			
24) Cambau 2015	10, 15, 18, 26-27, 28-31	clinical	139	139	MDR					139				
25) Hillemann 2008 & Beckert 2012	15		1	1	H37Rv ATCC 27294	gWT parental strain				1				
	15		5	5	pan-S	gWT parental strain				5				

The green line denotes the current WHO and CLSI CC for LZD DST in MGIT (1 mg/L). Notable limitation: Some laboratories are in common to Studies 15, 18-20 and 23-25.

5.4.2 LZD MICs for mutated isolates in MGIT

In vitro and clinical isolates

Hillemann *et al.* and Beckert *et al.* (study 25) conducted *in vitro* selection experiments using six different parental strains with MICs of ≤1 mg/L in MGIT (Table 66). Four isolates with the *rplC* C154R mutation were obtained, as well as five isolates with two distinct *rrl* mutations (G2299/2061T and G2814/2576T). The LZD MICs of these mutants, including one isolate for which the resistance mechanism could not be elucidated, were all 4-32 mg/L.

23 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Table 66. LZD MICs for in vitro mutants in MGIT.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	LZD MIC (mg/L) 0.5	1	2	4	8	16	32
25) Hillemann 2008 & Beckert 2012	*in vitro* mutants	1	H37Rv ATCC 27294	gWT parental strain		1					
		2		*rplC* C154R				2			
		1		mechanism unknown				1			
		5	pan-S	gWT parental strain		5					
		2		*rplC* C154R					2		
		4		*rrl* G2299/2061T							4
		1		*rrl* G2814/2576T						1	

The green line denotes the current WHO and CLSI CC for LZD DST in MGIT (1 mg/L).

Three studies with LZD MICs reported sequencing data for pNWT clinical isolates (Table 67). Richter *et al.* and Beckert *et al.* (Study 15) reported MICs of 4-8 mg/L for four resistant isolates that arose during treatment, compared to MICs of 0.5-1 mg/L for their respective parental strains. The three isolates with MICs of 8 mg/L were *rplC* C154R mutants. The fourth mutant did not have mutations in *rrl* (rplC was not sequenced). The MIC for the *rplC* C154R mutant from Perdigão *et al.* (Study 26) could have been either 2 or 4 mg/L, as 2 mg/L was not tested. Similarly, the MIC for the *rrl* A2810/2572C and G2814/2576T double mutant from Bloemberg *et al.* and Somoskovi *et al.* (Study 27) could have been either 8 or 16 mg/L, as 8 mg/L was not tested.

Table 67. LZD MICs for mutated clinical isolates in MGIT.

Studies	Isolate origin	Unique isolates	Type of isolates	Genotypic results	LZD MIC (mg/L) 0.25	0.5	1	2	4	8	16	32
15) Richter 2007 & Beckert 2012	clinical	4		gWT parental strain		**3**	1					
		3	MDR	*rplC* C154R						3		
		1		mechanism unknown					1			
26) Perdigão 2016	clinical	1	XDR	*rplC* C154R					1			
27) Bloemberg 2015 & Somoskovi 2015	clinical	1	XDR	*rrl* A2810/2572C & G2814/2576T							1	

The green line denotes the current WHO and CLSI CC for LZD DST in MGIT (1 mg/L).

5.3.3 Conclusion for LZD CC for MGIT

Additional, untruncated MIC distributions for pWT isolates with sequence data are needed to better define the degree of overlap between pWT and pNWT distributions and the variation between different laboratories and/or different datasets. In the absence of these data, **1 mg/L** was reaffirmed as the CC.

5.5 References for LZD MIC studies

1. Wang, J.Y. *et al.* Fluoroquinolone resistance in *Mycobacterium tuberculosis* isolates: associated genetic mutations and relationship to antimicrobial exposure. *J Antimicrob Chemother* 59, 860-5 (2007).

2. Weiss, T. *et al.* Low minimal inhibitory concentrations of linezolid against multidrug-resistant tuberculosis strains. *Eur Respir J* 45, 285-7 (2015).

3. Schön, T. *et al.* Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis. Int J Tuberc Lung Dis* 15, 502-9 (2011).

4. Ahmed, I., Jabeen, K., Inayat, R. & Hasan, R. Susceptibility testing of extensively drug-resistant and pre-extensively drug-resistant *Mycobacterium tuberculosis* against levofloxacin, linezolid, and amoxicillin-clavulanate. *Antimicrob Agents Chemother* 57, 2522-5 (2013).

5. McGee, B. *et al.* Population pharmacokinetics of linezolid in adults with pulmonary tuberculosis. *Antimicrob Agents Chemother* 53, 3981-4 (2009).

6. Koh, W.J. *et al.* Daily 300 mg dose of linezolid for the treatment of intractable multidrug-resistant and extensively drug-resistant tuberculosis. *J Antimicrob Chemother 64*, 388-91 (2009).

7. Alcalá, L. *et al. In vitro* activities of linezolid against clinical isolates of *Mycobacterium tuberculosis* that are susceptible or resistant to first-line antituberculous drugs. *Antimicrob Agents Chemother* 47, 416-7 (2003).

8. Yang, C. *et al. In vitro* activity of linezolid against clinical isolates of *Mycobacterium tuberculosis*, including multidrug-resistant and extensively drug-resistant strains from Beijing, China. *Jpn J Infect Dis* 65, 240-2 (2012).

9. (a) Pholwat, S., Heysell, S., Stroup, S., Foongladda, S. & Houpt, E. Rapid first- and second-line drug susceptibility assay for *Mycobacterium tuberculosis* isolates by use of quantitative PCR. *J Clin Microbiol* 49, 69-75 (2011).

(b) Pholwat, S., Ehdaie, B., Foongladda, S., Kelly, K. & Houpt, E. Real-time PCR using mycobacteriophage DNA for rapid phenotypic drug susceptibility results for *Mycobacterium tuberculosis. J Clin Microbiol* 50, 754-61 (2012).

(c) Pholwat, S. *et al.* Integrated microfluidic card with TaqMan probes and high-resolution melt analysis to detect tuberculosis drug resistance mutations across 10 genes. *MBio* 6, e02273 (2015).

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(b) Rey-Jurado, E., Tudo, G., Soy, D. & Gonzalez-Martin, J. Activity and interactions of levofloxacin, linezolid, ethambutol and amikacin in three-drug combinations against *Mycobacterium tuberculosis* isolates in a human macrophage model. *Int J Antimicrob Agents* 42, 524-30 (2013).

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(b) Beckert, P. *et al. rplC* T460C identified as a dominant mutation in linezolid-resistant *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* 56, 2743-5 (2012).

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(b) Beckert, P. *et al. rplC* T460C identified as a dominant mutation in linezolid-resistant *Mycobacterium tuberculosis* strains. *Antimicrob Agents Chemother* 56, 2743-5 (2012).

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(b) Somoskovi, A., Bruderer, V., Hömke, R., Bloemberg, G.V. & Böttger, E.C. A mutation associated with clofazimine and bedaquiline cross-resistance in MDR-TB following bedaquiline treatment. *Eur Respir J* 45, 554-7 (2015).

6. Delamanid

6.0 DLM MIC data stratification and current breakpoints

DLM MIC data were stratified based on mutations in the five known resistance genes: *ddn* (*Rv3547*), *fgd1* (*Rv0407*), *fbiA* (*Rv3261*), *fbiB* (*Rv3262*), and *fbiC* (*Rv1173*) (a detailed discussion of the known resistance mechanisms for this drug can be found in the supplement). Since many isolates had mutations in resistance genes, only those mutations that correlated with elevated MICs were shown in the tables (i.e. all other mutations were regarded as gWT).

No breakpoints for DLM testing have been defined by CLSI, FDA or WHO to date. In parallel with the marketing authorization by European Medicines Agency, EUCAST has designed 0.06 mg/L as a medium-independent CB for DLM testing.[24] Otsuka, meanwhile, has proposed 0.05 mg/L as the ECOFF and 0.2 mg/L as the CC for DLM testing on both 7H10 and 7H11.[25]

6.1 DLM MIC data on LJ

6.1.1 DLM MICs for pWT isolates on LJ

No studies presenting MIC distributions for pWT isolates on LJ were identified.

6.1.2 DLM MICs for mutated isolates on LJ

No studies with MIC distributions for mutated isolates on LJ were found.

6.1.3 Conclusion for DLM CC for LJ

Given that no MIC data were identified for LJ, a CC could not be defined.

6.2 DLM MIC data on 7H10

6.2.1 DLM MICs for pWT isolates on 7H10

One study was identified that reported DLM MIC data for 99 clinical isolates on 7H10 (Table 68). Dataset 1 included 24 baseline isolates from trial 102 and Dataset 2 consisted of 52 baseline isolates from trial 101. Dataset 3 featured 23 additional clinical isolates. With the exception of Dataset 1, which had a pWT MIC distribution of 0.006-0.025 mg/L (with a mode at 0.012 mg/L), modes could not be defined because the distributions were truncated at the lower end.

Table 68. DLM MICs for pWT isolates on 7H10.

						DLM MIC (mg/L)							
Studies	**Lab**	**Dataset**	**Isolate origin**	**Unique isolates**	**Type of isolates**	*0.003*	*0.006*	*0.012*	*0.025*	*0.05*	*0.06*	*0.01*	*0.2*
1) Diacon 2011 & Stinson 2016	1	1	clinical	23	pan-S		6	**16**	1				
	1	1		1	non-MDR			**1**					
	1	2		43	pan-S		21	19	1	2			
	1	2	clinical	7	non-MDR		5	2					
	1	2		2	MDR		2						
	1	3		1	H37Rv ATCC 25618		1						
	1	3		7	pan-S		4	3					
	1	3	clinical	10	MDR		7	3					
	1	3		6	XDR		4	2					

The purple line denotes the current EUCAST CB for DLM testing (0.06 mg/L).

24 European Committee for Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 7.1, valid from 2017-03-10. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.xls (accessed 9.4.2017).
25 Stinson, K. *et al.* MIC of delamanid (OPC-67683) against *Mycobacterium tuberculosis* clinical isolates and a proposed critical concentration. *Antimicrob Agents Chemother* 60, 3316-22 (2016).

6.2.2 DLM MICs for mutated isolates on 7H10

No studies presenting MICs for mutated isolates on 7H10 were identified.

6.2.3 Conclusion for DLM CC for 7H10

All 7H10 MICs for clinical isolates were from a single laboratory, which precluded an assessment of the inter-laboratory reproducibility of testing. Moreover, many MICs were truncated. Consequently, these data were deemed insufficient to define a CC.

6.3 DLM MIC data on 7H11

6.3.1 DLM MICs for pWT isolates on 7H11

Two studies were identified that reported DLM MIC data for the pWT population on 7H11 (Table 69). Gler *et al.* and Stinson *et al.* (Study 2) presented data from two laboratories. Dataset 4 featured 45 clinical isolates from Japan, whereas Dataset 5 contained 314 pWT baseline isolates from trial 204. Only one MIC was truncated and the modes of the distributions in both laboratories were 0.004 mg/L.

Schena *et al.* (Study 3) retested 13 baseline isolates from trial 204, which were also part of Dataset 5, and a 14th isolate provided by Otsuka that was not part of that trial. This is consequently not an independent dataset and could only provide information regarding the inter-laboratory reproducibility of MIC testing. The isolate that was not part of trial 204 had an unusually high MIC of 0.12 mg/L. The 13 isolates from trial 204 had MICs within the range of Study 2 (i.e. 0.002-0.016 mg/L), but had two modes (one at 0.004 and a second at 0.016 mg/L). However, the sample size of this study was small.

Table 69. DLM MICs for pWT isolates on 7H11.

							DLM MIC (mg/L)									
Studies	Lab	Dataset	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5
2) Gler 2012 & Stinson 2016	2	4		1	2	H37Rv ATCC 25618				**2**							
	2	4	clinical	37	37	MDR			3	**26**	8						
	2	4	clinical	8	8	XDR			1	**7**							
	3	5		1	50	H37Rv ATCC 25618			11	**32**	7						
	3	5		103	103	MDR		1	40	**54**	8						
	3	5	clinical	8	8	XDR			**5**	1	1		1				
	3	5	clinical	9	9	non-MDR			3	**5**	1						
	3	5	clinical	2	2	MDR			**2**								
	3	5	clinical	5	5	MDR				2	**3**						
	3	5	clinical	1	1	XDR					**1**						
	3	5	clinical	43	43	MDR			4	**31**	8						
	3	5	clinical	7	7	XDR			1	2	**4**						
	3	5		10	10	MDR			**6**	2	2						
	3	5	clinical	6	6	MDR			2	**4**							
	3	5	clinical	1	1	XDR				**1**							
	3	5	clinical	10	10	MDR			3	**6**	1						
	3	5	clinical	4	4	XDR			1	**3**							
	3	5	clinical	100	100	MDR			17	**58**	19	6					
	3	5	clinical	5	5	non-MDR			1	**2**	2						
3) Schena 2016	4	5a		1	1	H37Rv ATCC 27294						**1**					
	4	5a	clinical	14	14		gWT		1	**6**	1	**5**			1		

The purple line denotes the current EUCAST CB for DLM testing (0.06 mg/L). Notable limitation: 13 isolates from Dataset 5a represent a subset of Dataset 5.

6.3.2 DLM MICs for mutated isolates on 7H11

Clinical isolates

Two studies reported MICs for mutated isolates on 7H11 (Table 70). Gler *et al.* and Stinson *et al.* (Study 2) described two naturally resistant clinical isolates from Egypt and the Republic of Korea from trial 204. DLM resistance in these isolates must have evolved independently, as they harboured unique *ddn* mutations (L107P and a 43bp deletion, respectively).

Schena *et al.* (Study 3) retested the naturally resistant isolate with the *ddn* L107P mutation from Study 2 and confirmed an MIC of 1 mg/L. Four *ddn* mutants with three unique mutations that arose during treatment in trials NCT02573350 and NCT01131351 were also tested.

Table 70. DLM MICs for mutated clinical isolates on 7H11.

Studies	Lab	Dataset	Isolate origin	Unique isolates	Type of isolates	Genotypic results	DLM MIC (mg/L) 0.03	0.06	0.12	0.25	0.5	1	2	4	8	12.5	16	25
2) Gler 2012 & Stinson 2016	3	5	clinical	1	MDR	natural *ddn* L107P						1						
	3	5		1		natural *ddn* 43 bp deletion										1		
3) Schena 2016	4	5a	clinical	1	MDR	natural *ddn* L107P						1						
	4	5a		4		clinical *ddn* mutants (3 different mut.)						1						3

The purple line denotes the current EUCAST CB for DLM testing (0.06 mg/L). Notable limitation: The same *ddn* L107P mutant was tested in both studies.

6.3.3 Conclusion for DLM CC for 7H11

Most 7H11 MICs came from just two laboratories. Nevertheless, more than 350 unique pWT isolates were tested, and so an interim CC of **0.016 mg/L** was defined for this medium. All mutant isolates were detected at this CC.

6.4 DLM MIC data in MGIT

6.4.1 DLM MICs for pWT isolates in MGIT

Two studies were identified that reported DLM MIC data for the pWT population in MGIT (Table 71). Schena *et al.* (Study 4) included data from two laboratories. Laboratory 4 tested a total of 149 gWT isolates, which formed a pWT MIC distribution of 0.002-0.06 mg/L (with a mode at 0.016 mg/L). This included the 13 baseline isolates from trial 204 and a 14th isolate provided by Otsuka (Dataset 5a) that had also been tested by Schena *et al.* on 7H11 (Study 3). The remaining 135 isolates were clinical isolates from other sources (Dataset 6). 51 isolates from the latter set were retested in laboratory 5 using two different dilution series (Dataset 6a). The distribution of the 20 isolates that were not truncated had a mode at 0.008 mg/L. 95 additional, unique pWT isolates were tested using a limited dilution series, which resulted in a truncated distribution (Dataset 7).

Bloemberg *et al.* and Keller *et al.* (Study 5) used crushed DLM tablets rather than pure compound for MIC testing. They reported a pWT MIC distribution of 0.005-0.04 mg/L (with a mode at 0.01 mg/L) for 20 clinical isolates.

Table 71. DLM MICs for pWT isolates in MGIT.

Studies	Lab	Dataset	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	DLM MIC (mg/L) 0.001	0.002	0.0025	0.004	0.005	0.008	0.01	0.016	0.02	0.03	0.04	0.06	0.08	0.12	0.16
4) Schena 2016	4	5a	clinical	1	6	H37Rv ATCC 27294				1		**2**		**3**							
	4	5a		14	14		gWT				**5**		3		**5**		1					
	4	6		135	135		gWT		1		11		45		**47**		29		2			
	5	6a		20	20		gWT				3		**11**		3		2		1			
	5	6a	clinical	31	31		gWT								18		12		1			
	5	7		95	95										92		2		1			
5) Bloemberg 2015 & Keller 2015	6	8	clinical	1	1	H37Rv ATCC 27294							**1**								
	6	8		10	10	pan-S					1		**5**		3		1					
	6	8		10	10	MDR					1		**8**				1					
	6	8		1	2	XDR	gWT parent						**2**								

The purple line denotes the current EUCAST CB for DLM testing (0.06 mg/L). Notable limitations: Dataset 6a represents a subset of Dataset 6. Study 5 did not use pure DLM for MIC testing.

6.4.2 DLM MICs for mutated isolates in MGIT

Clinical isolates

The same two studies mentioned in Section 6.4.1 (Studies 4 and 5) included MICs for mutated isolates in MGIT (Table 72). In Dataset 5a, Schena *et al.* (Study 4) tested a naturally resistant *ddn* L107P mutant, as well as four *ddn* mutants that arose during the course of trials NCT02573350 and NCT01131351 (these had also been tested on 7H11 in Studies 2 and 3). Moreover, three additional naturally resistant MDR Beijing isolates, which all harboured a nonsense mutation at codon 88 of ddn, and a fourth naturally resistant XDR Beijing isolate with a mutation at codon 250 of *fbiA* were tested in laboratories 4 and 5 (Datasets 6 and 6a).

Bloemberg *et al.* and Keller *et al.* (Study 5), which did not use pure DLM for MIC testing, reported on the acquisition of DLM resistance during treatment. The identified *fbiA* D49T mutant had an MIC of >0.32 mg/L, compared to 0.01 mg/L for the gWT parent strain.

Table 72. DLM MICs for mutated clinical isolates in MGIT.

							DLM MIC (mg/L)																		
Studies	Lab	Dataset	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.005	0.01	0.02	0.04	0.06	0.08	0.12	0.16	0.25	0.32	0.4	0.5	1	1.56	2	4	6.25	8	16	25
	4	5a	clinical	1	1	MDR	natural *ddn* L107P									1											
	4	5a		4	4		clinical *ddn* mutants (3 different mut.)													1							3
	4	6		3	3	MDR	natural *ddn* W88Stop																				3
4) Schena 2016	4	6		1	1	XDR	natural *fbiA* K250Stop																				1
	5	6a	clinical	1	1		natural *ddn* W88Stop																	1			
	5	6a		2	2		natural *ddn* W88Stop														2						
	5	6a		1	1		natural *fbiA* K250Stop																	1			
5) Bloemberg 2015 & Keller 2015	6	8	clinical	1	2	XDR	gWT parent		2																		
	6	8		1	3	XDR	*fbiA* D49T											3									

The purple line denotes the current EUCAST CB for DLM testing (0.06 mg/L). Notable limitations: The isolates in Datasets 6 and 6a are identical. Study 5 did not use pure DLM for MIC testing.

6.4.3 Conclusion for DLM CC for MGIT

MGIT MICs were available from two laboratories that used pure compound and a third laboratory that relied on crushed DLM. Despite these methodological differences, the pWT MIC distributions were comparable and supported an interim CC of **0.06 mg/L**. As was the case for 7H11, more data are required to strengthen this conclusion for MGIT.

6.5 References for DLM MIC studies

1. (a) Diacon, A.H. *et al.* Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. *Int J Tuberc Lung Dis* 15, 949-54 (2011).

(b) Stinson, K. *et al.* MIC of delamanid (OPC-67683) against *Mycobacterium tuberculosis* clinical isolates and a proposed critical concentration. *Antimicrob Agents Chemother* 60, 3316-22 (2016).

2. (a) Gler, M.T. *et al.* Delamanid for multidrug-resistant pulmonary tuberculosis. *N Engl J Med* 366, 2151-2160 (2012)

(b) Stinson, K. *et al.* MIC of delamanid (OPC-67683) against *Mycobacterium tuberculosis* clinical isolates and a proposed critical concentration. *Antimicrob Agents Chemother* 60, 3316-22 (2016).

3. Schena, E. *et al.* Delamanid susceptibility testing of *Mycobacterium tuberculosis* using the resazurin microtitre assay and the BACTEC MGIT 960 system. *J Antimicrob Chemother* 71, 1532-9 (2016).

4. Schena, E. *et al.* Delamanid susceptibility testing of *Mycobacterium tuberculosis* using the resazurin microtitre assay and the BACTEC MGIT 960 system. *J Antimicrob Chemother* 71, 1532-9 (2016).

5. (a) Bloemberg, G.V. *et al.* Acquired resistance to bedaquiline and delamanid in therapy for tuberculosis. *N Engl J Med* 373, 1986-8 (2015).

(b) Keller, P.M. *et al.* Determination of MIC distribution and epidemiological cutoff values for bedaquiline and delamanid in *Mycobacterium tuberculosis* using the MGIT 960 System equipped with TB eXiST. *Antimicrob Agents Chemother* 59, 4352-5 (2015).

7. Fluoroquinolones

7.0 FQ MIC data stratification and current breakpoints

FQ MIC data were stratified based on mutations in *gyrA* (*Rv0006*) and *gyrB* (*Rv0005*) (details regarding these resistance mechanisms can be found in the supplement). For *gyrA*, only mutations within the QRDR spanning codons 74-109 were noted for this report, with the exception of T80A, A90G and S95T, which were not included in this report as these are considered natural polymorphisms.[26, 27, 28] The 1998 numbering system, as described by Maruri *et al.*, was used for *gyrB* mutations, as this is the same nomenclature employed by version 2 of the Hain Lifescience Genotype MTBDR*sl* assay. For *gyrB*, only mutations in codons 485-543 were reported (codons 500-540 represent the QRDR according to Pantel *et al.* 2012, and the MTBDR*sl* v2 assay interrogates codons 536-541).[29, 30] For Malik *et al.*, all mutations were reported, as this study generated allelic exchange mutants to specifically study the role of putative polymorphisms and mutations within and outside of the gyrase QRDR regions.[31] We excluded isolates with mutations in more than one resistance gene from the discussion, although these data are available in the supplementary MIC files.

Version 2 of the Hain GenoType MTBDR*sl* assay interrogates mutations in both genes, whereas version 1 includes only *gyrA* (the mutations that are specifically targeted by mutation probes by this assay are highlighted in **bold**, whereas mutations that merely inferred by lack of binding of a wild type probe are <u>underlined</u> in the tables of this report).

CLSI has set only CCs for the FQs, whereas WHO has also set CBs for MFX (Table 73).[32, 33]

Table 73. Overview of current FQ CCs and CBs.

	LJ		7H10		7H11		MGIT	
Drug	WHO	CLSI	WHO	CLSI	WHO	CLSI	WHO	CLSI
OFX	4.0	–	2.0		2.0		2.0	
LFX	–	–	1.0		–		1.5	
GFX	–	–	1.0	–	–		–	
MFX	–	–	0.5 & 2.0	0.5	–	0.5	0.5 & 2.0	0.25

Green CCs were set by both the WHO and CLSI; red CCs or CBs were set by WHO; blue CCs were set by CLSI. All concentrations are in mg/L.

26 Feuerriegel, S., Köser, C.U. & Niemann, S. Phylogenetic polymorphisms in antibiotic resistance genes of the *Mycobacterium tuberculosis* complex. *J Antimicrob Chemother* 69, 1205-10 (2014).
27 Ajileye, A. *et al.* Some synonymous and nonsynonymous *gyrA* mutations in *Mycobacterium tuberculosis* lead to systematic false-positive fluoroquinolone resistance results with the Hain GenoType MTBDR*sl* assays. *Antimicrob Agents Chemother* 61, e02169-16 (2017).
28 Maruri, F. *et al.* A systematic review of gyrase mutations associated with fluoroquinolone-resistant *Mycobacterium tuberculosis* and a proposed gyrase numbering system. *J Antimicrob Chemother* 67, 819-31 (2012).
29 Pantel, A. *et al.* Extending the definition of the *GyrB* quinolone resistance-determining region in *Mycobacterium tuberculosis* DNA gyrase for assessing fluoroquinolone resistance in *M. tuberculosis*. *Antimicrob Agents Chemother* 56, 1990-6 (2012).
30 Tagliani, E. *et al.* Diagnostic performance of the new version of GenoType MTBDR*sl* (V2.0) assay for detection of resistance to fluoroquinolones and second line injectable drugs: a multicenter study. *J Clin Microbiol* 53, 2961-9 (2015).
31 Malik, S., Willby, M., Sikes, D., Tsodikov, O.V. & Posey, J.E. New insights into fluoroquinolone resistance in *Mycobacterium tuberculosis*: functional genetic analysis of *gyrA* and *gyrB* mutations. *PLoS One* 7, e39754 (2012).
32 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).
33 World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1 (accessed 13.8.2015).

7.A.1 OFX MIC data on LJ

7.A.1.1 OFX MICs for pWT isolates on LJ

Six studies from three laboratories were identified that reported OFX MIC data for the pWT population on LJ (Table 74). Most distributions were truncated at the lower end and Fabry *et al.* (Study 5) tested a non-standard dilution range. The insight into the shape of the pWT MIC distribution was therefore limited, but the current CC of 4 mg/L appeared to be appropriate.

Table 74. OFX MICs for pWT isolates on LJ.

						OFX MIC (mg/L)							
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**5**	**8**	**10**	**32**
1) Rigouts 2016	1	clinical	187		gWT	12	**85**	73	9		4	4	
2) Coeck 2016	1	clinical	45		gWT	2	**18**	12	6		1	6	
3) Barletta 2014	1	clinical	11	MDR	gWT	1		**8**	2				
4) Vincent 2012	1	clinical	7	different levels of R	gWT		**2**	**2**	1			2	
5) Fabry 1995	2	clinical	19					**19**					
6) Sulochana 1999	3	clinical	55	enriched for OFX R			25	5	2	23			

The red line denotes the current WHO CC for OFX on LJ (4 mg/L). Notable limitations: Studies 1-4 were conducted in the same laboratory and Study 6 was enriched for isolates resistant to OFX.

7.A.1.2 OFX MICs for mutated isolates on LJ

gyrA mutants

Clinical isolates

67 clinical isolates from the same laboratory, harbouring *gyrA* mutations that are targeted by the MTBDR*sl* assays, were tested on LJ medium (Table 75). Based on the current OFX CC for this medium, only 3 of these isolates (4% (95% CI, 1-13%) were phenotypically OFX-susceptible. 100% (95% CI, 29-100%) of these OFX-susceptible mutants had *gyrA* A90V or D94A mutations.

Table 75. OFX MICs for clinical gyrA mutants on LJ.

						OFX MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**10**
1) Rigouts 2016	1	clinical	1		*gyrA* A74S			1			
1) Rigouts 2016	1	clinical	3		*gyrA* D89N						3
1) Rigouts 2016	1	clinical	13		*gyrA* **A90V**				1	7	5
2) Coeck 2016	1	clinical	7		*gyrA* **A90V**				1	4	2
4) Vincent 2012	1	clinical	1	different levels of R	*gyrA* **A90V**						1
1) Rigouts 2016	1	clinical	4		*gyrA* **S91P**					3	1
2) Coeck 2016	1	clinical	2		*gyrA* **S91P**					2	
1) Rigouts 2016	1	clinical	6		*gyrA* **D94A**			1		4	1
2) Coeck 2016	1	clinical	5		*gyrA* **D94A**					4	1
1) Rigouts 2016	1	clinical	6		*gyrA* **D94G**						6
2) Coeck 2016	1	clinical	14		*gyrA* **D94G**					1	13
4) Vincent 2012	1	clinical	1	different levels of R	*gyrA* **D94G**						1
2) Coeck 2016	1	clinical	1		*gyrA* **D94H**						1
2) Coeck 2016	1	clinical	1		*gyrA* D94N						1
4) Vincent 2012	1	clinical	1	different levels of R	*gyrA* D94N						1
1) Rigouts 2016	1	clinical	1		*gyrA* **A90V, S91P**						1
4) Vincent 2012	1	clinical	1	different levels of R	*gyrA* **A90V**, D94Y						1

The red line denotes the current WHO CC for OFX DST on LJ (4 mg/L). Notable limitation: All studies were conducted in the same laboratory.

gyrB mutants

Clinical isolate

Only a single *gyrB* mutant was tested on LJ and found to be resistant to OFX, which would be interpreted as conferring resistance with the MTBDR*sl* v2 assay (Table 76).

Table 76. OFX MICs for clinical gyrB mutant on LJ.

				OFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**10**
1) Rigouts 2016	clinical	1	*gyrB* N538S						1

The red line denotes the current WHO CC for OFX DST on LJ (4 mg/L).

7.A.1.3 Conclusion for OFX CC for LJ

Although the identified MIC data were limited, the current OFX CC of **4 mg/L** was maintained given the importance of LJ testing in some settings. Low-level *gyrA* mutants can be misclassified at this concentration because of the inherent variation in testing. **However, it should be noted that testing of OFX is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific FQs used in treatment regimens.**

7.A.2 OFX MIC data on 7H10

7.A.2.1 OFX MICs for pWT isolates on 7H10

10 studies from nine laboratories were identified that reported OFX MIC data for the pWT population on 7H10 (Table 77). Some variation in testing was apparent, as the modes of the distributions varied between 0.25 and 1 mg/L. The current CC of 2 mg/L therefore appeared to be appropriate.

7.A.2.2 OFX MICs for mutated isolates on 7H10

gyrA mutants

Allelic exchange results

Malik *et al.* (Study 13) generated allelic exchange mutants using either H37Rv, Erdman or CDC1551 (Table 78). The *gyrA* A74S, T80A, G247S, and A384V mutations (or combinations thereof) did not change the OFX MICs significantly compared to the parent strains. This also applied to the A90G mutation, which has been noted to cause a systematic-false positive result with the MTBDRs*l* assays.[34] In contrast, the increase for the low-level A90V mutants were significant (i.e. >2 doubling dilutions), but spanned the CC (with reported MICs of 2-8 mg/L). This suggested that the MIC of A90V was close to the CC, which likely results in poor DST reproducibility for isolates with this mutation due to the normal technical variation inherent in MIC testing. This was not the case for the high-level D94G mutants, which had OFX MICs of between 8 and >16 mg/L.

Table 78. OFX MICs for gyrA allelic exchange mutants on 7H10.

						OFX MIC (mg/L)							
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
13) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT		**2**						
		1	2		*gyrA* A74S, **D94G**							1	1
		1	2		*gyrA* T80A	2							
		1	2		*gyrA* T80A, A90G	2							
		1	2		*gyrA* **A90V**				1	1			
		1	2		*gyrA* G247S		2						
		1	2		*gyrA* A384V			2					
		1	2	Erdman	gWT		**2**						
		1	2		*gyrA* A74S			1	1				
		1	2		*gyrA* T80A		2						
		1	2		*gyrA* T80A, A90G	2							
		1	2		*gyrA* A90G	2							
		1	2		*gyrA* **A90V**				1		1		
		1	2		*gyrA* G247S		2						
		1	2		*gyrA* A384V			2					
		1	2	CDC1551	*gyrA* **D94G**						2		

The green line denotes the current WHO and CLSI CC for OFX DST on 7H10 (2 mg/L).

34 Ajileye, A. *et al.* Some synonymous and nonsynonymous *gyrA* mutations in *Mycobacterium tuberculosis* lead to systematic false-positive fluoroquinolone resistance results with the Hain GenoType MTBDRs*l* assays. *Antimicrob Agents Chemother* 61, e02169-16 (2017).

Table 77. OFX MICs for pWT isolates on 7H10.

							OFX MIC (mg/L)																		
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.06**	**0.12**	**0.15**	**0.25**	**0.3**	**0.5**	**0.6**	**1**	**1.2**	**2**	**2.5**	**4**	**6**	**8**	**16**	**32**	**64**	**128**	**256**
7) Wang 2007	4	clinical	420	420	different levels of R		2	2		10		116		**243**		40		3		2	2				
8) Angeby 2010	5		1	4	H37Rv ATCC 27294							**4**													
	5	clinical	109	109	mostly pan-S			1		7		**75**		25						1					
9) Niward 2016	5		2	2	H37Rv ATCC 27294					**1**		**1**													
	5	clinical	48	48		gWT				**26**		19				1		1		1					
10) Pholwat 2011, 2012 & 2015	6		1	1	H37Rv ATCC 27294							**1**													
	6	clinical	31	31	different levels of R	gWT				4		**20**		6		1									
11) Rey-Jurado 2012	7		1	1	H37Rv							**1**													
	7	clinical	10	10	pan-S					1		**9**													
	7	clinical	12	12	INH-R									**12**											
12) Karak 1995	8	clinical	61	61	H37Rv & different levels of R				2		**32**		27												
13) Malik 2012	9		1	2	H37Rv							**2**													
	9		1	2	Erdman							**2**													
	9		1	2	H37Rv							**2**													
	9		1	2	Erdman							**2**													
	9		1	2	H37Rv							**2**													
	9		1	2	Erdman							**2**													
14) Blackman 2012, Devasia 2012,	10		1	1	H37Rv ATCC 27294									**1**											
van der Heijden 2013, Eilertson 2014	10	clinical	17	17		gWT								**11**							1		1	2	2
15) Farhat 2015 & 2016	11	clinical	147	147	MDR or XDR	gWT								131		3		7	5	1					
16) Hu 2013	12		1	1	H37Rv ATCC 25618									1											

The green line denotes the current WHO and CLSI CC for OFX DST on 7H10 (2 mg/L). Notable limitation: Studies 8 and 9 were conducted in the same laboratory.

Clinical isolates

Six studies were identified with 166 MICs for isolates with *gyrA* mutations that are targeted by the MTBDRs*l* assays (Table 79). Based on the current OFX CC for this medium, 34 of these MICs were below the current CC (20% (95% CI, 15-27%)). 17 of these 'susceptible' results (50% (95% CI, 32-68%) were due to the *gyrA* A90V and D94A mutations. Methodological variation in MIC testing likely accounted for these results, in line the allelic exchange data for *gyrA* A90V.

gyrB mutants

Allelic exchange results

Malik *et al.* (Study 13) generated allelic exchange mutants using H37Rv and Erdman (Table 80). The OFX MICs of some of these *gyrB* mutants were elevated above the current OFX CC (e.g. D500H mutants had MICs 4-8 mg/L in both genetic backgrounds). However, the majority of OFX MICs for *gyrB* mutants were 0.5-2 mg/L, and so these isolates were consequently OFX-susceptible based on the current OFX CC of 2 mg/L, as were the gWT control strains (MICs of 0.5 mg/L). These isolates included several mutants that would be interpreted as OFX-resistant by the MTBDRs*l* v2 assay (e.g. N538K). However, the OFX MICs for several of these 'LPA-resistant' isolates were equal to the CC, suggesting that these mutations only result in modest MIC increases.

Table 80. OFX MICs for gyrB allelic exchange mutants on 7H10.

						OFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
13) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT		**2**				
		1	2		*gyrB* V340L		2				
		1	2		*gyrB* R485C			2			
		1	2		*gyrB* R485C, T539N					1	1
		1	2		*gyrB* D500A				2		
		1	2		*gyrB* D500H					1	1
		1	2		*gyrB* D500N					2	
		1	2		*gyrB* D533A		2				
		1	2		*gyrB* **N538D**					2	
		1	2		*gyrB* **N538D**, T546M				2		
		1	2		*gyrB* N538K				2		
		1	2		*gyrB* N538T, T546M		2				
		1	2		*gyrB* T539N				2		
		1	2		*gyrB* T539P		1	1			
		1	2		*gyrB* E540D		2				
		1	2		*gyrB* **E540V**					2	
		1	2		*gyrB* A543T		1		1		
		1	2		*gyrB* A543V			2			
		1	2		*gyrB* T546M		2				
		1	2	Erdman	gWT		**2**				
		1	2		*gyrB* M330I		2				
		1	2		*gyrB* V340L			2			
		1	2		*gyrB* R485C			2			
		1	2		*gyrB* R485C, T539N						2
		1	2		*gyrB* D500A				2		
		1	2		*gyrB* D500H					1	1
		1	2		*gyrB* D500N					2	
		1	2		*gyrB* D533A			2			
		1	2		*gyrB* **N538D**					2	
		1	2		*gyrB* **N538D**, T546M					2	
		1	2		*gyrB* N538K				2		
		1	2		*gyrB* N538T, T546M		2				
		1	2		*gyrB* T539N				2		
		1	2		*gyrB* T539P		1	1			
		1	2		*gyrB* E540D		1	1			
		1	2		*gyrB* **E540V**					2	
		1	2		*gyrB* A543T			2			
		1	2		*gyrB* A543V				2		
		1	2		*gyrB* T546M		2				

The green line denotes the current WHO and CLSI CC for OFX DST on 7H10 (2 mg/L).

Table 79. OFX MICs for clinical gyrA mutants on 7H10.

						OFX MIC (mg/L)												
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**6**	**8**	**10**	**16**	**32**	**50**	**64**	**128**	**256**
9) Niward 2016	clinical	1	1		*gyrA* A74S		1											
9) Niward 2016	clinical	1	1		*gyrA* D89N				1									
15) Farhat 2015 & 2016	clinical	3	3	MDR or XDR	*gyrA* D89N		2		1									
9) Niward 2016	clinical	9	9		*gyrA* **A90V**		1	5	3									
13) Malik 2012	clinical	1	2	different levels of R	*gyrA* **A90V**				2									
14) Blackman 2012, Devasia 2012,	clinical	2	2		*gyrA* **A90V**						1							1
15) Farhat 2015 & 2016	clinical	23	23	MDR or XDR	*gyrA* **A90V**		2		19	1		1						
16) Hu 2013	clinical	8	8		*gyrA* **A90V**			2			2		4					
14) Blackman 2012, Devasia 2012,	clinical	1	1		*gyrA* **S91P**											1		
15) Farhat 2015 & 2016	clinical	2	2	MDR or XDR	*gyrA* **S91P**				2									
16) Hu 2013	clinical	6	6		*gyrA* **S91P**		1	2			1		2					
9) Niward 2016	clinical	1	1		*gyrA* **D94A**						1							
15) Farhat 2015 & 2016	clinical	12	12	MDR or XDR	*gyrA* **D94A**		1	6	4		1							
9) Niward 2016	clinical	8	8		*gyrA* **D94G**				1		6		1					
10) Pholwat 2011, 2012 & 2015	clinical	4	4	different levels of R	*gyrA* **D94G**				1		3							
13) Malik 2012	clinical	1	2	different levels of R	*gyrA* **D94G**						2							
14) Blackman 2012, Devasia 2012,	clinical	8	8		*gyrA* **D94G**						1					5		2
15) Farhat 2015 & 2016	clinical	26	26	MDR or XDR	*gyrA* **D94G**		4	1	7	10	2	1	1					
16) Hu 2013	clinical	15	15		*gyrA* **D94G**			2			1		9	2	1			
9) Niward 2016	clinical	2	2		*gyrA* D94N				1		1							
14) Blackman 2012, Devasia 2012,	clinical	2	2		*gyrA* D94N						1						1	
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrA* D94N					1								
16) Hu 2013	clinical	9	9		*gyrA* D94N			3			1		5					
9) Niward 2016	clinical	2	2		*gyrA* D94Y				1		1							
10) Pholwat 2011, 2012 & 2015	clinical	1	1	different levels of R	*gyrA* D94Y				1									
14) Blackman 2012, Devasia 2012,	clinical	2	2		*gyrA* D94Y											2		
15) Farhat 2015 & 2016	clinical	5	5	MDR or XDR	*gyrA* D94Y				2	1	1		1					
16) Hu 2013	clinical	1	1		*gyrA* A74S, **D94G**								1					
16) Hu 2013	clinical	1	1		*gyrA* A74S, D94N			1										
9) Niward 2016	clinical	1	1		*gyrA* **A90V**, D94N									1				
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrA* **S91P**, **D94G**			1										
9) Niward 2016	clinical	2	2		*gyrA* **S91P**, D94Y						2							
14) Blackman 2012, Devasia 2012,	clinical	3	3		*gyrA* **D94G**, D94N											1		2

The green line denotes the current WHO and CLSI CC for OFX DST on 7H10 (2 mg/L).

Clinical isolates

Excluding the *gyrB* V340L mutation, which does not confer FQ resistance based on the aforementioned allelic exchange results, 12 isolates from four studies had *gyrB* mutations (Table 81). These included eight isolates that would be considered resistant by the MTBDRs*l* v2 assay, of which two were susceptible (25% (95% CI, 3-65%)).

Table 81. OFX MICs for clinical gyrB mutants on 7H10.

						OFX MIC (mg/L)								
Studies	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.25	0.5	1	2	4	8	16	32	64
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* V340L			2						
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrB* R485H			1						
10) Pholwat 2011, 2012 & 2015	clinical	1	1	different levels of R	*gyrB* S486F					1				
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrB* S486Y			1						
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* D500H						2			
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* **N538D**					2				
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrB* **N538D**					1				
14) Blackman 2012, Devasia 2012,	clinical	1	1		*gyrB* N538I									1
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrB* T539A					1				
15) Farhat 2015 & 2016	clinical	1	1	MDR or XDR	*gyrB* E540D			1						
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* R485C, T539N					1	1			
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* **N538D**, T546M					2				
13) Malik 2012	clinical	1	2	different levels of R	*gyrB* N538T, T546M		2							

The green line denotes the current WHO and CLSI CC for OFX DST on 7H10 (2 mg/L).

7.A.2.3 Conclusion for OFX CC for 7H10

The current CC of **2 mg/L** was reaffirmed based upon the identified MIC data, but it was apparent that because of the variation in testing known *gyrA* resistance mutations, particularly those that confer low-level resistance, can be misclassified as susceptible. **However, it should be noted that testing of OFX is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific FQs used in treatment regimens.**

7.A.3 OFX MIC data on 7H11

7.A.3.1 OFX MICs for pWT isolates on 7H11

Four studies were identified that reported OFX MIC data for the pWT population on 7H11 (Table 82). Given the truncations and lack of genotypic data for some studies, insights into the shape of the pWT MIC distributions were limited.

Table 82. OFX MICs for pWT isolates on 7H11.

						OFX MIC (mg/L)								
Studies	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.25	0.5	1	2	4	5	8	16	32
17) Fattorini 1999 &		1	1	H37Rv ATCC 27294				**1**						
Giannoni 2005	clinical	10	10		gWT		**3**	2	**3**			1	1	
18) Rodríguez 2001	clinical	55	55				34	10	10	1				
19) Bernard 2016		1	6	H37Rv			2	**4**						
20) Sulochana 1999	clinical	55	55	enriched for OFX R				18	12	1	24			

The green line denotes the current WHO and CLSI CC for OFX DST on 7H11 (2 mg/L). Notable limitation: Study 20 was enriched for OFX-resistant isolates.

7.A.3.2 OFX MICs for mutated isolates on 7H11

gyrA mutants

Clinical isolates

44 isolates from two studies tested on 7H10 harboured *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 83). Based on the current OFX CC, two of these mutants (5% (95% CI, 1-15%) were phenotypically OFX-susceptible.

Table 83. OFX MICs for mouse and clinical gyrA mutants on 7H11.

				OFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**16**
19) Bernard 2016	mice	2	*gyrA* D89G	1		1			
19) Bernard 2016	mice	6	*gyrA* D89N				3	3	
17) Fattorini 1999 & Giannoni 2005	clinical	9	*gyrA* **A90V**				2	7	
19) Bernard 2016	mice	2	*gyrA* **A90V**					1	1
17) Fattorini 1999 & Giannoni 2005	clinical	2	*gyrA* **S91P**				1	1	
19) Bernard 2016	mice	2	*gyrA* **S91P**				1		1
17) Fattorini 1999 & Giannoni 2005	clinical	1	*gyrA* **D94A**				1		
19) Bernard 2016	mice	1	*gyrA* **D94A**				1		
17) Fattorini 1999 & Giannoni 2005	clinical	4	*gyrA* **D94G**					4	
19) Bernard 2016	mice	7	*gyrA* **D94G**					2	5
19) Bernard 2016	mice	2	*gyrA* **D94H**						2
17) Fattorini 1999 & Giannoni 2005	clinical	1	*gyrA* D94N					1	
19) Bernard 2016	mice	2	*gyrA* D94N						2
19) Bernard 2016	mice	3	*gyrA* D94Y					1	2

The green line denotes the current WHO and CLSI CC for OFX DST on 7H11 (2 mg/L).

gyrB mutants

Mouse isolates

12 *gyrB* mutants that arose during the treatment of mice with LFX were tested on 7H11, of which two had mutations that are targeted by the MTBDR*sl* v2 assay (Table 84). One of these isolates was susceptible at the current CC (50% (95% CI, 1-99%)).

Table 84. OFX MICs for mouse gyrB mutants on 7H11.

				OFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**16**
		1	*gyrB* R485C		1				
		1	*gyrB* D500A			1			
		4	*gyrB* D500H					4	
19) Bernard 2016	mice	2	*gyrB* D500N				1	1	
		1	*gyrB* N538T				1		
		1	*gyrB* E540A		1				
		2	*gyrB* A543V			1	1		

The green line denotes the current WHO and CLSI CC for OFX DST on 7H11 (2 mg/L).

7.A.3.3 Conclusion for OFX CC for 7H11

It was difficult to define the upper end of the pWT MIC distribution due to the truncations of MICs and a lack of supporting genetic data for some studies. Nevertheless, the current CC of **2 mg/L** was maintained. **However, it should be noted that testing of OFX is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific FQs used in treatment regimens.**

7.A.4 OFX MIC data in MGIT

7.A.4.1 OFX MICs for pWT isolates in MGIT

12 studies were identified with OFX MIC data for pWT isolates tested in MGIT (Table 85). Based on their results, Cambau *et al.* (Study 30) suggested that the current CC should be lowered from 2 to 1 mg/L. Assessing this proposal was complicated by the fact that the pWT MIC distributions from most of the remaining studies, including Rüsch-Gerdes *et al.* (Study 28) and Rodrigues *et al.* (Study 29), which are cited in the CLSI guidelines in support of the current CC of 2 mg/L, were truncated.[35] This was compounded by the lack of genotypic data for several studies.

Table 85. OFX MICs for pWT isolates in MGIT.

							OFX MIC (mg/L)											
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**2.5**	**4**	**5**	**6**	**8**	**10**	**16**	**32**	**50**
21) Sturegård 2015	16		2	2	H37Rv ATCC 27294			**1**	**1**										
	16	clinical	26	26				**16**	7	1		1					1		
22) Alvarez 2014	17		1	1	H37Rv				**1**										
	17	clinical	5	5	MDR or XDR	gWT						3			2				
23) Sirgel 2012	18		1	6	H37Rv ATCC 27294			6											
	18	clinical	125	125	different levels of R	gWT		106	13	5					1				
24) Kam 2006	19	clinical	108	108	MDR			102	6										
	19		4	4	MDR	gWT						2			2				
25) Kambli 2015	20	clinical	1	1	H37Rv ATCC 27294			1											
	20		30	30		gWT		26	2	2									
26) Rigouts	1		1	5	H37Rv ATCC 27294				**5**										
	1	clinical	9	9		gWT		7	2										
27) Zheng 2016	12		1	1	H37Rv ATCC 25618			1											
	12	clinical	207	207	MDR			111	70	7		2			3		12	1	1
28) Rusch-Gerdes	21-23	clinical	10	30	H37Rv ATCC 27294 & pan-S				30										
2006	21-23		21	63	different levels of R			27	24		12								
29) Rodrigues 2008	20	clinical	1	1	H37Rv ATCC 27294				1										
	20		10	10	pan-S				10										
	20		20	20	different levels of R				18	2									
30) Cambau 2015	21, 24-31	clinical	114	114	MDR				114										
	21, 24-31		3	3	MDR	gWT				3									
31) Sharma 2011	32	clinical	36	36	different levels of R				24	1		4	7						
32) Springer 2009	24	clinical	12	12						11						1			

The green line denotes the current WHO and CLSI CC for OFX DST in MGIT (2 mg/L). Notable limitations: Studies 25 and 29 were conducted in the same laboratory. Some laboratories were in common to Studies 28, 30 and 32. The genotypic results in Study 25 were based on the MTBDR*sl* v1.

7.A.4.2 OFX MICs for mutated isolates in MGIT

gyrA mutants

Clinical isolates

262 isolates from six studies had *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 86). Of these, only four mutants (2% (95% CI, 0-4%) were susceptible to OFX. Given that these four isolates, of which two (50% (95% CI, 7-93%)) were *gyrA* A90V or D94A mutants, had MICs equal to the CC (2 mg/L), methodological variation in MIC testing likely accounted for these results.

35 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Table 86. OFX MICs for clinical gyrA mutants in MGIT.

					OFX MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**1**	**1.5**	**2**	**4**	**6**	**8**	**10**	**16**	**50**
23) Sirgel 2012	clinical	1	different levels of R	*gyrA* G88C							1		
23) Sirgel 2012	clinical	1	different levels of R	*gyrA* D89G			1						
26) Rigouts	clinical	1		*gyrA* D89N			1						
30) Cambau 2015	clinical	1	MDR	*gyrA* D89N							1		
22) Alvarez 2014	clinical	2	MDR or XDR	*gyrA* **A90V**				2					
23) Sirgel 2012	clinical	12	different levels of R	*gyrA* **A90V**			1	8	3				
24) Kam 2006	clinical	8	MDR	*gyrA* **A90V**				6		1		1	
25) Kambli 2015	clinical	25		*gyrA* **A90V**				22		3			
26) Rigouts	clinical	19		*gyrA* **A90V**				13	6				
30) Cambau 2015	clinical	3	MDR	*gyrA* **A90V**							3		
22) Alvarez 2014	clinical	1	MDR or XDR	*gyrA* **S91P**								1	
23) Sirgel 2012	clinical	3	different levels of R	*gyrA* **S91P**				2	1				
24) Kam 2006	clinical	1	MDR	*gyrA* **S91P**				1					
25) Kambli 2015	clinical	6		*gyrA* **S91P**				5		1			
26) Rigouts	clinical	5		*gyrA* **S91P**				5					
30) Cambau 2015	clinical	5	MDR	*gyrA* **S91P**							5		
23) Sirgel 2012	clinical	7	different levels of R	*gyrA* **D94A**				3	1	2	1		
24) Kam 2006	clinical	5	MDR	*gyrA* **D94A**				4		1			
25) Kambli 2015	clinical	4		*gyrA* **D94A**				1		3			
26) Rigouts	clinical	11		*gyrA* **D94A**			1	9	1				
30) Cambau 2015	clinical	2	MDR	*gyrA* **D94A**							2		
22) Alvarez 2014	clinical	4	MDR or XDR	*gyrA* **D94G**						2		2	
23) Sirgel 2012	clinical	17	different levels of R	*gyrA* **D94G**					1	6	9		1
24) Kam 2006	clinical	12	MDR	*gyrA* **D94G**						8		4	
25) Kambli 2015	clinical	42		*gyrA* **D94G**						35	6	1	
26) Rigouts	clinical	20		*gyrA* **D94G**				3	10	4	3		
30) Cambau 2015	clinical	7	MDR	*gyrA* **D94G**							6	1	
24) Kam 2006	clinical	1	MDR	*gyrA* **D94H**						1			
25) Kambli 2015	clinical	3		*gyrA* **D94H**						3			
26) Rigouts	clinical	1		*gyrA* **D94H**							1		
30) Cambau 2015	clinical	1	MDR	*gyrA* **D94H**							1		
23) Sirgel 2012	clinical	6	different levels of R	*gyrA* D94N				1			5		
26) Rigouts	clinical	1		*gyrA* D94N							1		
30) Cambau 2015	clinical	1	MDR	*gyrA* D94N								1	
24) Kam 2006	clinical	2	MDR	*gyrA* D94Y						1		1	
26) Rigouts	clinical	1		*gyrA* D94Y						1			
23) Sirgel 2012	clinical	2	different levels of R	*gyrA* **A90V, D94G**				1		1			
30) Cambau 2015	clinical	1	MDR	*gyrA* **A90V, D94G**								1	
24) Kam 2006	clinical	2	MDR	*gyrA* **A90V**, P102H				2					
23) Sirgel 2012	clinical	2	different levels of R	*gyrA* **D94G**, D94N						1			1
25) Kambli 2015	clinical	13		*gyrA* D94N or D94Y						10	2	1	

The green line denotes the current WHO and CLSI CC for OFX DST in MGIT (2 mg/L). Notable limitation: The genotypic results in Study 25 were based on the MTBDR*sl* v1.

gyrB mutants

Clinical isolates

Three isolates from one study had *gyrB* mutations that are targeted by the MTBDR*sl* v2 assay (Table 87). Two of these mutants were susceptible, with an MIC of 1.5 mg/L (67% (95% CI, 9-99%)).

Table 87. OFX MICs for clinical gyrB mutants in MGIT.

				OFX MIC (mg/L)							
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**1.5**	**2**	**4**	**6**	**8**	**10**
26) Rigouts	clinical	1	*gyrB* N538S								1
		1	*gyrB* T539A			1					
		1	*gyrB* T539N			1					

The green line denotes the current WHO and CLSI CC for OFX DST in MGIT (2 mg/L).

7.A.4.3 Conclusion for OFX CC for MGIT

Based on the data from their multi-centre investigation, Cambau *et al.* (Study 30) proposed that the current CC for MGIT should be lowered from 2 mg/L to 1 mg/L.[36] As almost all identified studies reported truncated MIC distributions, little is known about the shape of the pWT MIC distribution in MGIT. This finding was compounded by the fact that some key studies lacked sequencing data, which would have helped to define the upper end of the pWT MIC distribution. In the end, the current CC of **2 mg/L** was maintained, both based on the pWT MIC data and the fact that only 2% (95% CI, 0-4%) of *gyrA* mutants were misclassified as susceptible at this concentration. **However, it should be noted that testing of OFX is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific FQs used in treatment regimens.**

7.A.5 References for OFX MIC studies

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2. Coeck, N. *et al.* Correlation of different phenotypic drug susceptibility testing methods for four fluoroquinolones in *Mycobacterium tuberculosis. J Antimicrob Chemother* 71, 1233-40 (2016).

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(c) Pholwat, S. *et al.* Integrated microfluidic card with TaqMan probes and high-resolution melt analysis to detect tuberculosis drug resistance mutations across 10 genes. *MBio* 6, e02273 (2015).

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36 Cambau, E. *et al.* Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother* **70**, 686-96 (2015).

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30. Cambau, E. *et al.* Revisiting susceptibility testing in MDR-TB by a standardized quantitative phenotypic assessment in a European multicentre study. *J Antimicrob Chemother* 70, 686-96 (2015).

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7.B.1 LFX MIC data on LJ

7.B.1.1 LFX MICs for pWT isolates on LJ

Only one study was identified that reported LFX MIC data for the pWT MIC distribution on LJ (Table 88).

Table 88. LFX MICs for pWT isolates on LJ.

				LFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**12**
1) Coeck 2016	clinical	56	gWT	20	18	5	7	3	3

7.B.1.2 LFX MICs for mutated isolates on LJ

***gyrA* mutants**

Clinical isolates

34 isolates from a single study by Coeck *et al.* (Study 1) harboured *gyrA* mutations that are targeted by the MTBDR*sl* assays and were tested on LJ medium (Table 89). Only a single mutant with a *gyrA* D94A mutation has MICs of ≤2 mg/L.

Table 89. LFX MICs for clinical gyrA mutants on LJ.

				LFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**12**
1) Coeck 2016	clinical	1	*gyrA* D89N				1		
		9	*gyrA* **A90V**				5	4	
		3	*gyrA* **S91P**				3		
		5	*gyrA* **D94A**			1	3	1	
		14	*gyrA* **D94G**				1	9	4
		1	*gyrA* **D94H**						1
		1	*gyrA* D94N					1	

gyrB mutants

Clinical isolates

MIC data was found for just one clinical *gyrB* mutant, which would be interpreted as resistant using the MTBDRs*l* v2 assay (Table 90).

Table 90. LFX MICs for clinical gyrB mutants on LJ.

				LFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.5**	**1**	**2**	**4**	**8**	**12**
1) Coeck 2016	clinical	1	*gyrB* N538S						1

7.B.1.3 Conclusion for LFX CC for LJ

LJ represents the only medium available for DST in many high-TB burden settings. Therefore, an interim CC of **2 mg/L** was set even though only a single study was identified for this drug-medium combination. This concentration was chosen as it corresponds to half the OFX CC on LJ (OFX consists of equal amounts of the active L-isomer of OFX (i.e. LFX) and the largely inactive D-isomer, which means that LFX is about twice as potent as OFX).[37] This concentration was also in line with the limited pWT and pNWT MIC data presented by Coeck *et al.* (Study 1).

7.B.2 LFX MIC data on 7H10

7.B.2.1 LFX MICs for pWT isolates on 7H10

Eight studies were identified, including Sanders *et al.* (Study 7), which has been cited in the CLSI guidelines in support of the current CC of 1 mg/L, that reported LFX MIC data for the pWT population on 7H10 (Table 91).[38] The modes of these distributions varied between 0.12 and 0.5 mg/L and thus supported the current CC.

Table 91. LFX MICs for pWT isolates on 7H10.

							LFX MIC (mg/L)									
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**4.5**	**8**	**16**
2) Wang 2007	2	clinical	420	420	different levels of R		3	17	139	**216**	39	3	1		2	
3) Angeby 2010	3		1	4	H37Rv ATCC 27294				**4**							
	3	clinical	109	109	mostly pan-S			3	**82**	23			1			
4) Niward 2016	3		2	2	H37Rv ATCC 27294			**1**	**1**							
	3	clinical	48	48		gWT		**23**	21		3				1	
5) Peloquin 2008	4		1	1	H37Rv ATCC 27294					**1**						
	4	clinical	10	10					1	**8**	1					
6) Willby 2015	5	clinical	46	46		gWT			12	**29**	5					
7) Sanders 2004	6	clinical	44	44					3	**22**	7		1	11		
8) Malik 2012	5		1	2	H37Rv				2							
	5		1	2	Erdman				2							
	5		1	2	H37Rv				2							
	5		1	2	Erdman				2							
	5		1	2	H37Rv				2							
	5		1	2	Erdman				2							
9) Hu 2013	7		1	1	H37Rv ATCC 25618						1					

The green line denotes the current WHO and CLSI CC for LFX DST on 7H10 (1 mg/L). Notable limitations: Studies 3 and 4, and Studies 6 and 8 were conducted in the same laboratory, respectively.

37 World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis (2014). http://apps.who.int/iris/bitstream/10665/130918/1/9789241548809_eng.pdf?ua=1&ua=1 (accessed 13.8.2015).
38 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

7.B.2.2 LFX MICs for mutated isolates on 7H10

gyrA mutants

Allelic exchange results

Malik *et al.* (Study 8) generated allelic exchange mutants using H37Rv, Erdman and CDC1551 (Table 92). The *gyrA* A74S, T80A, G247S, and A384V mutations (or combinations thereof) did not change the LFX MICs significantly (i.e. no MICs for these mutants were above the current LFX CC of 1 mg/L). This also applied to isolates with the A90G mutation, which has been noted to cause a systematic-false positive result with the MTBDR*sl* assays.[39] As seen for OFX, the LFX MICs of the A90V mutants spanned the CC (with reported MICs of 0.5-4 mg/L), whereas the D94G mutants had LFX MICs of 8-16 mg/L.

Table 92. LFX MICs for gyrA allelic exchange mutants on 7H10.

						LFX MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
8) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT	2						
		1	2		*gyrA* A74S, **D94G**							2
		1	2		*gyrA* T80A	2						
		1	2		*gyrA* T80A, A90G	2						
		1	2		*gyrA* **A90V**		1		1			
		1	2		*gyrA* G247S	2						
		1	2		*gyrA* A384V		2					
		1	2	Erdman	gWT	2						
		1	2		*gyrA* A74S			2				
		1	2		*gyrA* T80A	2						
		1	2		*gyrA* T80A, A90G	2						
		1	2		*gyrA* A90G	2						
		1	2		*gyrA* **A90V**		1			1		
		1	2		*gyrA* G247S	2						
		1	2		*gyrA* A384V		2					
		1	2	CDC1551	*gyrA* **D94G**						2	

The green line denotes the current WHO and CLSI CC for LFX DST on 7H10 (1 mg/L).

39 Ajileye, A. *et al.* Some synonymous and nonsynonymous *gyrA* mutations in *Mycobacterium tuberculosis* lead to systematic false-positive fluoroquinolone resistance results with the Hain GenoType MTBDR*sl* assays. *Antimicrob Agents Chemother* 61, e02169-16 (2017).

Clinical isolates

150 isolates from four studies that were tested in three laboratories had *gyrA* mutations that are targeted by the MTBDRs*l* assays (Table 93). Based on the current CC, 18 (12% (95% CI, 7-18%) of these mutants were phenotypically LFX-susceptible. Eight of these 'susceptible' isolates (44% (95% CI, 22-69%) harboured *gyrA* A90V or D94A mutations.

Table 93. LFX MICs for clinical gyrA mutants on 7H10.

						LFX MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
4) Niward 2016	3	clinical	1	1	*gyrA* A74S		1					
9) Hu 2013	7	clinical	1	1	*gyrA* A74S				1			
8) Malik 2012	5	clinical	1	2	*gyrA* T80A	2						
6) Willby 2015	5	clinical	1	1	*gyrA* G88C						1	
6) Willby 2015	5	clinical	1	1	*gyrA* D89G			1				
4) Niward 2016	3	clinical	1	1	*gyrA* D89N				1			
6) Willby 2015	5	clinical	1	1	*gyrA* D89N					1		
4) Niward 2016	3	clinical	9	9	*gyrA* **A90V**	1		5	3			
6) Willby 2015	5	clinical	24	24	*gyrA* **A90V**				7	15	2	
8) Malik 2012	5	clinical	1	2	*gyrA* **A90V**				2			
9) Hu 2013	7	clinical	8	8	*gyrA* **A90V**			2		2	4	
6) Willby 2015	5	clinical	1	1	*gyrA* **S91P**				1			
9) Hu 2013	7	clinical	6	6	*gyrA* **S91P**			3		1	2	
4) Niward 2016	3	clinical	1	1	*gyrA* **D94A**					1		
6) Willby 2015	5	clinical	12	12	*gyrA* **D94A**				6	4	2	
4) Niward 2016	3	clinical	8	8	*gyrA* **D94G**				1	6	1	
6) Willby 2015	5	clinical	25	25	*gyrA* **D94G**				1	1	18	5
8) Malik 2012	5	clinical	1	2	*gyrA* **D94G**						2	
9) Hu 2013	7	clinical	15	15	*gyrA* **D94G**			2		1	9	3
6) Willby 2015	5	clinical	11	11	*gyrA* D94N					1	9	1
4) Niward 2016	3	clinical	2	2	*gyrA* D94N				1	1		
9) Hu 2013	7	clinical	9	9	*gyrA* D94N			3		1	5	
4) Niward 2016	3	clinical	2	2	*gyrA* D94Y				1	1		
6) Willby 2015	5	clinical	2	2	*gyrA* D94Y					1	1	
8) Malik 2012	5	clinical	1	2	*gyrA* G247S	2						
9) Hu 2013	7	clinical	1	1	*gyrA* A74S, **D94G**						1	
9) Hu 2013	7	clinical	1	1	*gyrA* A74S, D94N			1				
6) Willby 2015	5	clinical	1	1	*gyrA* **A90V, S91P**						1	
6) Willby 2015	5	clinical	1	1	*gyrA* **A90V, D94G**						1	
4) Niward 2016	3	clinical	1	1	*gyrA* **A90V**, D94N							1
6) Willby 2015	5	clinical	1	1	*gyrA* **S91P, D94G**						1	
4) Niward 2016	3	clinical	2	2	*gyrA* **S91P**, D94Y					2		
6) Willby 2015	5	clinical	1	1	*gyrA* G88C, **A90V, D94G**				1			

The green line denotes the current WHO and CLSI CC for LFX DST on 7H10 (1 mg/L). Notable limitation: Studies 6 and 8 were conducted in the same laboratory.

gyrB mutants

Allelic exchange results

Malik *et al.* (Study 8) generated allelic exchange mutants using H37Rv and Erdman (Table 94). The LFX MICs of some of these *gyrB* mutants were elevated above the current LFX CC (e.g. D500H mutants had MICs 2-4 mg/L in both genetic backgrounds tested). However, the majority of LFX MICs were ≤0.25-1 mg/L, and were consequently LFX-susceptible based on the current LFX CC of 1 mg/L, as were the gWT control strains (MICs of ≤0.25 mg/L). These isolates included several mutants that would be interpreted as resistant by the MTBDR*sl* v2 assay (e.g. N538K). However, the LFX MICs for several of these 'LPA-resistant' isolates were equal to the CC, suggesting that these mutations may only result in modest MIC increases.

Table 94. LFX MICs for gyrB allelic exchange mutants on 7H10.

						LFX MIC (mg/L)				
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**
8) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT	2				
		1	2		*gyrB* V340L	2				
		1	2		*gyrB* R485C			2		
		1	2		*gyrB* R485C, T539N				1	1
		1	2		*gyrB* D500A			2		
		1	2		*gyrB* D500H				1	1
		1	2		*gyrB* D500N				2	
		1	2		*gyrB* D533A	2				
		1	2		*gyrB* **N538D**				2	
		1	2		*gyrB* **N538D**, T546M				2	
		1	2		*gyrB* N538K			2		
		1	2		*gyrB* N538T, T546M		2			
		1	2		*gyrB* T539N			2		
		1	2		*gyrB* T539P		1	1		
		1	2		*gyrB* E540D		2			
		1	2		*gyrB* **E540V**			1	1	
		1	2		*gyrB* A543T			2		
		1	2		*gyrB* A543V		1	1		
		1	2		*gyrB* T546M	2				
		1	2	Erdman	gWT	2				
		1	2		*gyrB* M330I	2				
		1	2		*gyrB* V340L		2			
		1	2		*gyrB* R485C			2		
		1	2		*gyrB* R485C, T539N					2
		1	2		*gyrB* D500A			2		
		1	2		*gyrB* D500H				1	1
		1	2		*gyrB* D500N				2	
		1	2		*gyrB* D533A	2				
		1	2		*gyrB* **N538D**				2	
		1	2		*gyrB* **N538D**, T546M				2	
		1	2		*gyrB* N538K			2		
		1	2		*gyrB* N538T, T546M		2			
		1	2		*gyrB* T539N			2		
		1	2		*gyrB* T539P		1	1		
		1	2		*gyrB* E540D		2			
		1	2		*gyrB* **E540V**				2	
		1	2		*gyrB* A543T			2		
		1	2		*gyrB* A543V			2		
		1	2		*gyrB* T546M	2				

The green line denotes the current WHO and CLSI CC for LFX DST on 7H10 (1 mg/L).

Clinical isolates

11 isolates from two studies featured *gyrB* mutants that were tested on 7H10 (Table 95). One of the six isolates with mutations that are targeted by the MTBDR*sl* v2 assay tested susceptible on two occasions (17% (95% CI, 0-64%).

Table 95. LFX MICs for clinical gyrB mutants on 7H10.

						LFX MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
8) Malik 2012	5	clinical	1	2	*gyrB* R485C, T539N				2			
6) Willby 2015	5	clinical	1	1	*gyrB* D500H					1		
8) Malik 2012	5	clinical	1	2	*gyrB* D500H					2		
6) Willby 2015	5	clinical	2	2	*gyrB* D500N				2			
6) Willby 2015	5	clinical	1	1	*gyrB* **N538D**				1			
8) Malik 2012	5	clinical	1	2	*gyrB* **N538D**				2			
6) Willby 2015	5	clinical	1	1	*gyrB* N538K				1			
8) Malik 2012	5	clinical	1	2	*gyrB* R485C, T539N				2			
8) Malik 2012	5	clinical	1	2	*gyrB* **N538D**, T546M				2			
8) Malik 2012	5	clinical	1	2	*gyrB* N538T, T546M		2					

The green lines denote the current WHO and CLSI CC for LFX DST on 7H10 (1 mg/L). Notable limitation: Studies 6 and 8 were conducted in the same laboratory.

7.B.2.3 Conclusion for LFX CC for 7H10

The current CC of **1 mg/L** was reaffirmed, but it was noted that because of variation in testing certain *gyrA* mutants can be misclassified as susceptible at this concentration.

7.B.3 LFX MIC data on 7H11

7.B.3.1 LFX MICs for pWT isolates on 7H11

Four studies reported LFX MIC data for the pWT population on 7H11 (Table 96). Two of these studies reported MICs for at least 10 isolates.

Table 96. LFX MICs for pWT isolates on 7H11.

						LFX MIC (mg/L)									
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
10) Rodríguez 2002	clinical	243	243	mostly pan-S		2	25	**106**	104	1		1	1	2	1
11) Giannoni 2005	clinical	1	1	H37Rv ATCC 27294			**1**								
		10	10		gWT		1	**3**	1	**3**		1	1		
12) Rey-Jurado 2013a, Rey-Jurado 2013b, López-Gavín 2015 & López-Gavín 2016	clinical	1	1	H37Rv					**1**						
		11	11	pan-S					**11**						
		9	9	MDR					**9**						
13) Bernard 2016		1	6	H37Rv				2	**4**						

7.B.3.2 LFX MICs for mutated isolates on 7H11

gyrA mutants

Clinical isolates

Two studies featured MICs on 7H11 for 44 isolates with *gyrA* mutations that are targeted by the MTBDR*s*l assays (Table 97). 42 of these isolates (96% (95% CI, 85-99%) had MICs >1 mg/L.

Table 97. LFX MICs for mouse and clinical gyrA mutants on 7H11.

				LFX MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
13) Bernard 2016	mice	2	*gyrA* D89G	1		1				
13) Bernard 2016	mice	6	*gyrA* D89N				3	3		
11) Giannoni 2005	clinical	9	*gyrA* **A90V**				1	7	1	
13) Bernard 2016	mice	2	*gyrA* **A90V**					1	1	
11) Giannoni 2005	clinical	2	*gyrA* **S91P**				1	1		
13) Bernard 2016	mice	2	*gyrA* **S91P**				1	1		
11) Giannoni 2005	clinical	1	*gyrA* **D94A**					1		
13) Bernard 2016	mice	1	*gyrA* **D94A**				1			
11) Giannoni 2005	clinical	4	*gyrA* **D94G**				1	3		
13) Bernard 2016	mice	7	*gyrA* **D94G**					1	6	
13) Bernard 2016	mice	2	*gyrA* **D94H**						2	
11) Giannoni 2005	clinical	1	*gyrA* D94N				1			
13) Bernard 2016	mice	2	*gyrA* D94N						2	
13) Bernard 2016	mice	3	*gyrA* D94Y					1	2	

gyrB mutants

Mouse isolates

12 *gyrB* mutants that arose during the treatment of mice with LFX were tested on 7H11. Two of these isolates had mutations that are targeted by the MTBDR*s*l v2 assay (Table 98), with MICs that ranged from 0.5 to 2 mg/L.

Table 98. LFX MICs for mouse gyrB mutants on 7H11.

				LFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
		1	*gyrB* R485C		1				
		1	*gyrB* D500A			1			
		4	*gyrB* D500H					4	
13) Bernard 2016	mice	2	*gyrB* D500N				1	1	
		1	*gyrB* N538T				1		
		1	*gyrB* E540A		1				
		2	*gyrB* A543V			1	1		

7.B.3.3 Conclusion for LFX CC for 7H11

Given that only two studies were identified with more than 10 pWT isolates tested on 7H11, no CC was set for this medium.

7.B.4 LFX MIC data in MGIT

7.B.4.1 LFX MICs for pWT isolates in MGIT

12 studies from 10 laboratories reported LFX MICs for the pWT population in MGIT (Table 99). The shape of most pWT distributions, including for Lin *et al.* (Study 22) which has been cited in the CLSI guidelines in support of the current CC, could not be assessed because of truncations.[40] Where modes were identifiable, they ranged from 0.25 to 0.5 mg/L, which consequently pointed to 1 mg/L as the CC as opposed to the current concentration of 1.5 mg/L.

Table 99. LFX MICs for pWT isolates in MGIT.

							LFX MIC (mg/L)											
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.06	0.12	0.18	0.25	0.37	0.5	0.75	1	1.5	2	3	4	4.5	8	16
14) Nosova 2013 & Zimenkov 2013	12		1	1	H37Rv ATCC 25618							1									
	12	clinical	14	14	different levels of R			2		7		4				1					
	12		23	23	different levels of R	gWT		1		17		4		1							
15) Sturegård 2015	13		1	2	H37Rv ATCC 27294							2									
	13	clinical	20	20				1		5		13		1							
16) Sanders 2004	6	clinical	44	44				5				18		9				5	7		
17) Tessema 2017	14	clinical	41	41		gWT			11		27		3								
18) Heyckendorf 2017	14		1	1	H37Rv ATCC 27294								1								
	14	clinical	16	16	MDR or XDR	gWT			11		5										
19) Zheng 2016	7		1	1	H37Rv ATCC 25618					1											
	7	clinical	207	207	MDR					112		69		7		2		2		13	2
20) Werngren	15	clinical	12	12	different levels of R					9		2					1				
21) Alvarez 2014	16		1	1	H37Rv									1							
	16	clinical	5	5	MDR or XDR	gWT										3		1		1	
22) Lin 2009	17		1	3	H37Rv ATCC 27294							3									
	6		1	14								14									
	6	clinical	33	33										19		1	13				
23) Kambli 2015	18		1	1	H37Rv ATCC 27294						1										
	18	clinical	30	30		gWT					25		5								
24) Rigouts	1		1	5	H37Rv ATCC 27294						5										
	1	clinical	7	7		gWT					7										
25) Lin	6		73	73		gWT									66	1	2	4			

The green line denotes the current WHO and CLSI CC for LFX DST in MGIT (1.5 mg/L). Notable limitations: Studies 16, 22 and 25, and Studies 17 and 18 were conducted in the same laboratory, respectively. The genotypic results for Study 14 were based on a combination of sequencing and a microarray, whereas Study 23 relied on the MTBDRs/ v1.

40 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

7.B.4.2 LFX MICs for mutated isolates in MGIT

gyrA mutants

Clinical isolates

Five studies featured MICs for 241 *gyrA* mutants that would be interpreted as resistant by the MTBDRs*l* assays (Table 100). Using the current CC of 1.5 mg/L, 29 of these mutants would be misclassified as susceptible (12% (95% CI, 8-17%). Lowering the CC to 1 mg/L would likely improve the detection of at least some of these 'susceptible' mutants, given that 28 (97% (95% CI, 82-100%) had MICs of 1.12-1.5 mg/L.

Table 100. LFX MICs for clinical gyrA mutants in MGIT.

					LFX MIC (mg/L)															
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.75**	**1**	**1.12**	**1.5**	**2**	**3**	**4**	**4.5**	**6**	**7.5**	**8**	**12**	**16**	**32**	**64**
14) Nosova 2013 &	clinical	2	different levels of R	*gyrA* G88A					2										
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* G88C											1				
24) Rigouts	clinical	1		*gyrA* D89N			1												
14) Nosova 2013 &	clinical	10	different levels of R	*gyrA* **A90V**					5		3				2				
21) Alvarez 2014	clinical	2	MDR or XDR	*gyrA* **A90V**							1				1				
23) Kambli 2015	clinical	25		*gyrA* **A90V**				5		20									
24) Rigouts	clinical	18		*gyrA* **A90V**	1			8		8		1							
25) Lin	clinical	15		*gyrA* **A90V**					9	5	1								
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* **S91P**					1										
21) Alvarez 2014	clinical	1	MDR or XDR	*gyrA* **S91P**											1				
23) Kambli 2015	clinical	6		*gyrA* **S91P**				1		5									
24) Rigouts	clinical	5		*gyrA* **S91P**			1	2		2									
25) Lin	clinical	2		*gyrA* **S91P**					1	1									
14) Nosova 2013 &	clinical	6	different levels of R	*gyrA* **D94A**					6										
23) Kambli 2015	clinical	4		*gyrA* **D94A**				3					1						
24) Rigouts	clinical	11		*gyrA* **D94A**				7		4									
25) Lin	clinical	6		*gyrA* **D94A**					4	2									
14) Nosova 2013 &	clinical	13	different levels of R	*gyrA* **D94G**							8				3		2		
21) Alvarez 2014	clinical	4	MDR or XDR	*gyrA* **D94G**							1				3				
23) Kambli 2015	clinical	42		*gyrA* **D94G**						6			34			2			
24) Rigouts	clinical	19		*gyrA* **D94G**						10		7	2						
25) Lin	clinical	15		*gyrA* **D94G**					1	1	13								
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* **D94H**							1								
23) Kambli 2015	clinical	3		*gyrA* **D94H**						1			2						
24) Rigouts	clinical	1		*gyrA* **D94H**									1						
25) Lin	clinical	4		*gyrA* **D94H**						1	3								
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* D94N											1				
24) Rigouts	clinical	1		*gyrA* D94N										1					
25) Lin	clinical	1		*gyrA* D94N							1								
23) Kambli 2015	clinical	13		*gyrA* D94N or D94Y						2			10			1			
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* D94Y							1								
24) Rigouts	clinical	1		*gyrA* D94Y								1							
25) Lin	clinical	1		*gyrA* D94Y							1								
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* **A90V, D94G**															1
14) Nosova 2013 &	clinical	1	different levels of R	*gyrA* **A90V**, D94Y					1										
14) Nosova 2013 &	clinical	2	different levels of R	*gyrA* **S91P**, D94N							2								

The green line denotes the current WHO and CLSI CC for LFX DST in MGIT (1.5 mg/L). Notable limitations: The genotypic results for Study 14 were based on a combination of sequencing and a microarray, whereas Study 23 relied on the MTBDRs*l* v1.

gyrB mutants

Clinical isolates

Four studies from three laboratories reported MGIT MICs for 13 *gyrB* mutants (Table 101). This included five isolates that would be considered resistant using the MTBDRs*l* v2 assay, yet four of the resulting MICs (67% (95% CI, 22-96%)) were susceptible at the current CC. The two MICs for the *gyrB* N538D mutant were 1 and 2 mg/L, which suggested that this mutation conferred MICs close to the CC, resulting in poor reproducibility of testing.

Table 101. LFX MICs for clinical gyrB mutants in MGIT.

							LFX MIC (mg/L)											
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.18	0.37	0.5	0.75	1	1.12	1.5	2	3	4	4.5	6	8
14) Nosova 2013 & Zimenkov 2013	12	clinical	1	1	different levels of R	*gyrB* R485H					1								
17) Tessema 2017	14	clinical	3	3		*gyrB* R485H				3									
17) Tessema 2017	14	clinical	1	1		*gyrB* S486Y				1									
14) Nosova 2013 & Zimenkov 2013	12	clinical	1	1	different levels of R	*gyrB* D500H										1			
14) Nosova 2013 & Zimenkov 2013	12	clinical	1	2	different levels of R	*gyrB* **N538D**					1			1					
24) Rigouts	1	clinical	1	1		*gyrB* N538S												1	
24) Rigouts	1	clinical	1	1		*gyrB* T539A				1									
24) Rigouts	1	clinical	1	1		*gyrB* T539N				1									
17) Tessema 2017	14	clinical	1	1		*gyrB* E540D		1											
18) Heyckendorf 2017	14	clinical	2	2	MDR or XDR	*gyrB* A543V		1		1									

The green line denotes the current WHO and CLSI CC for LFX DST in MGIT (1.5 mg/L). Notable limitations: Studies 17 and 18 were conducted in the same laboratory. The genotypic results for Study 14 were based on a combination of sequencing and a microarray.

7.B.4.3 Conclusion for LFX CC for MGIT

The combined MIC data supported **1 mg/L** as the CC, as opposed to the current breakpoint of 1.5 mg/L. The CC was lowered accordingly.

7.B.5 References for LFX MIC studies

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7.C.1 GFX MIC data on LJ

7.C.1.1 GFX MICs for pWT isolates on LJ

Five studies were identified with GFX MIC data for the pWT population on LJ (Table 102). The MIC distributions were truncated at the lower end and only came from two laboratories. Somasundaram *et al.* (Study 1) was enriched for OFX-resistant isolates.

Table 102. GFX MICs for pWT isolates on LJ.

						GFX MIC (mg/L)					
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**
1) Somasundaram 2006	1	clinical	50	enriched for OFX R		1	8	**21**	1	8	11
2) Aung 2014 & Rigouts 2016	2	clinical	208		gWT		160	33	4	3	8
3) Coeck 2016	2	clinical	45		gWT		27	10	2	2	4
4) Barletta 2014	2	clinical	11	MDR	gWT		11				
5) Vincent 2012	2	clinical	7	different levels of R	gWT		**4**	1	2		

Notable limitations: Studies 2-5 were conducted in the same laboratory. Study 1 was enriched for resistant isolates.

7.C.1.2 GFX MICs for mutated isolates on LJ

gyrA mutants

Clinical isolates

137 isolates from the same laboratory harboured *gyrA* mutations that are targeted by the MTBDRs*l* assays (Table 103). Six of these mutants (5% (95% CI, 2-9%)) had MICs ≤0.5 mg/L. 100% (95% CI, 54-100%) of these isolates had *gyrA* A90V or D94A mutations.

Table 103. GFX MICs for clinical gyrA mutants on LJ.

						GFX MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
2) Aung 2014 & Rigouts 2016	2	clinical	2		*gyrA* A74S		2					
2) Aung 2014 & Rigouts 2016	2	clinical	2		*gyrA* G88C				1		1	
2) Aung 2014 & Rigouts 2016	2	clinical	1		*gyrA* D89G			1				
2) Aung 2014 & Rigouts 2016	2	clinical	4		*gyrA* D89N				1			3
2) Aung 2014 & Rigouts 2016	2	clinical	32		*gyrA* **A90V**	1	2	15	13			1
3) Coeck 2016	2	clinical	7		*gyrA* **A90V**		1	5	1			
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **A90V**					1		
2) Aung 2014 & Rigouts 2016	2	clinical	8		*gyrA* **S91P**				2	6		
3) Coeck 2016	2	clinical	2		*gyrA* **S91P**				2			
2) Aung 2014 & Rigouts 2016	2	clinical	18		*gyrA* **D94A**	1	1	9	5	2		
3) Coeck 2016	2	clinical	5		*gyrA* **D94A**			2	3			
2) Aung 2014 & Rigouts 2016	2	clinical	31		*gyrA* **D94G**			1	5	18	5	2
3) Coeck 2016	2	clinical	14		*gyrA* **D94G**			1	7	6		
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **D94G**						1	
2) Aung 2014 & Rigouts 2016	2	clinical	1		*gyrA* **D94H**					1		
3) Coeck 2016	2	clinical	1		*gyrA* **D94H**				1			
2) Aung 2014 & Rigouts 2016	2	clinical	2		*gyrA* D94N					1	1	
3) Coeck 2016	2	clinical	1		*gyrA* D94N					1		
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* D94N				1			
2) Aung 2014 & Rigouts 2016	2	clinical	2		*gyrA* D94Y					1	1	
2) Aung 2014 & Rigouts 2016	2	clinical	1		*gyrA* **A90V, S91P**							1
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **A90V**, D94Y					1		
2) Aung 2014 & Rigouts 2016	2	clinical	1		*gyrA* **D94G**, D94N					1		

Notable limitation: All studies were conducted in the same laboratory.

gyrB mutants

Clinical isolates

Seven clinical isolates from a single study had *gyrB* mutations (Table 104). Of the five isolates with mutations that are targeted by the MTBDR*sl* v2 assay, one (20% (95% CI, 1-72%)) had MICs ≤0.5 mg/L.

Table 104. GFX MICs for clinical gyrB mutants on LJ.

				GFX MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
2) Aung 2014 & Rigouts 2016	clinical	2	*gyrB* V535M		1	1				
		1	*gyrB* N538S							1
		1	*gyrB* T539A			1				
		1	*gyrB* T539I				1			
		2	*gyrB* T539N		1		1			

7.C.1.3 Conclusion for GFX CC for LJ

The pWT MICs distributions on LJ were severely truncated and only came from two laboratories. Despite these limitations, the consensus was to set **0.5 mg/L** as an interim CC given that many high-TB burden settings use LJ for DST. Additional data from well-designed studies will be necessary to re-evaluate this CC. It was noted that because of the inherent variation in testing some *gyrA* mutants were misclassified at this concentration.

7.C.2 GFX MIC data on 7H10

7.C.2.1 GFX MICs for pWT isolates on 7H10

A single study with just 10 clinical isolates was identified with GFX MICs on 7H10 (Table 105).

Table 105. GFX MICs for pWT isolates on 7H10.

				GFX MIC (mg/L)							
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**0.03**	**0.05**	**0.1**	**0.2**	**0.4**	**0.8**	**1**	**2**
6) Peloquin 2008		1	H37Rv ATCC 27294			**1**					
	clinical	10				1	**9**				

The red line denotes the current WHO CC for OFX DST on 7H10 (1 mg/L).

7.C.2.2 GFX MICs for mutated isolates on 7H10

No studies were found with MICs for mutants on 7H10.

7.C.2.3 Conclusion for GFX CC for 7H10

A single study with just 10 clinical isolates was identified by this review and no data were available for gNWT populations. The current GFX CC of 1 mg/L was therefore withdrawn.

7.C.3 GFX MIC data on 7H11

7.C.3.1 GFX MICs for pWT isolates on 7H11

Three studies were identified that reported GFX MIC data for pWT isolates on 7H11 (Table 106). Rodriguez *et al.* (Study 7) featured a substantial number of MICs that were not truncated. Somasundaram *et al.* (Study 8) was enriched for OFX-resistant isolates (20 of the 50 isolates tested were resistant to OFX using 2 mg/L as the CC with the absolute concentration method on LJ).

Table 106. GFX MICs for pWT isolates on 7H11.

					GFX MIC (mg/L)										
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**	**16**	**32**
7) Rodríguez 2002	clinical	243	mostly pan-S			13	**106**	100	18			3		2		1
8) Somasundaram 2006	clinical	50	enriched for OFX R					9	**21**	3	17					
9) Giannoni 2005		1	H37Rv ATCC 27294			**1**										
	clinical	10		gWT		2	**3**	1	2	1		1				

Notable limitation: Study 8 was enriched for resistant isolates.

7.C.3.2 GFX MICs for mutated isolates on 7H11

gyrA mutants

Clinical isolates

17 clinical isolates from a single study Giannoni *et al.* (Study 9) harboured *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 107). The MICs were 0.5-2 mg/L.

Table 107. GFX MICs for clinical gyrA mutants on 7H11.

				GFX MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
9) Giannoni 2005	clinical	9	*gyrA* **A90V**					1	6	2		
		2	*gyrA* **S91P**					1	1			
		1	*gyrA* **D94A**					1				
		4	*gyrA* **D94G**					1	2	1		
		1	*gyrA* D94N						1			

7.C.3.3 Conclusion for GFX CC for 7H11

Rodriguez *et al.* (Study 7) was the only study that featured a substantial number of MICs that were not truncated. The available data were considered insufficient to set a CC.

7.C.4 GFX MIC data in MGIT

7.C.4.1 GFX MICs for pWT isolates in MGIT

Two studies used MGIT to measure the GFX MICs for pWT isolates in MGIT (Table 108). The untruncated MIC distribution in Isaeva *et al.*, Nosova *et al.* & Zimenkov *et al.* (Study 10), which included MICs for 32 unique isolates, was 0.06-0.25 mg/L (with a mode at 0.12 mg/L).

Table 108. GFX MICs for pWT isolates in MGIT.

						GFX MIC (mg/L)									
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.03**	**0.06**	**0.12**	**0.18**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
10) Isaeva 2013, Nosova 2013 & Zimenkov 2013	clinical	1	2	H37Rv ATCC 25618				**1**		**1**					
		9	9	different levels of R			2	**7**							
		23	26	different levels of R	gWT		10	**14**		2					
11) Rigouts	clinical	1	5	H37Rv ATCC 27294				5							
		8	8		gWT			8							

Notable limitation: Isolates in Study 10 were interrogated using a combination of sequencing and microarray.

7.C.4.2 GFX MICs for mutated isolates in MGIT

gyrA mutants

Clinical isolates

Two studies from two laboratories featured 98 clinical isolates with *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 109). 10 of these isolates (10% (95% CI, 5-18%)) had MICs ≤0.25 mg/L. 100% (95% CI, 69-100%) of these had *gyrA* A90V or D94A mutations.

Table 109. GFX MICs for clinical gyrA mutants in MGIT.

						GFX MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.12**	**0.18**	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	2	2	different levels of R	*gyrA* G88A					2				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	2	different levels of R	*gyrA* G88C							1	1	
11) Rigouts	clinical	1	1		*gyrA* D89N					1				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	10	11	different levels of R	*gyrA* **A90V**				8	2		1		
11) Rigouts	clinical	19	19		*gyrA* **A90V**	1		5	13					
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* **S91P**				1					
11) Rigouts	clinical	4	4		*gyrA* **S91P**				2	2				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	6	7	different levels of R	*gyrA* **D94A**				6	1				
11) Rigouts	clinical	11	11		*gyrA* **D94A**			4	6	1				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	13	13	different levels of R	*gyrA* **D94G**					7		6		
11) Rigouts	clinical	21	21		*gyrA* **D94G**				7	14				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* **D94H**					1				
11) Rigouts	clinical	1	1		*gyrA* **D94H**					1				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* D94N							1		
11) Rigouts	clinical	1	1		*gyrA* D94N						1			
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* D94Y					1				
11) Rigouts	clinical	1	1		*gyrA* D94Y					1				
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* **A90V, D94G**								1	
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* **A90V**, D94Y				1					
10) Isaeva 2013, Nosova 2013 & Zimenkov	clinical	1	1	different levels of R	*gyrA* **S91P**, D94N							1		

Notable limitation: Isolates in Study 10 were interrogated using a combination of sequencing and microarray.

gyrB mutants

Clinical isolates

Two studies tested a total of six clinical *gyrB* mutants in MGIT (Table 110). Of the four mutants that would be regarded as GFX-resistant using the MTBDR*sl* v2 assay, two (50% (95% CI, 7-93%) had MICs ≤0.25 mg/L.

Table 110. GFX MICs for clinical gyrB mutants in MGIT.

						GFX MIC (mg/L)										
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.03**	**0.06**	**0.12**	**0.18**	**0.25**	**0.5**	**1**	**1.5**	**2**	**4**	**8**
10) Isaeva 2013,	clinical	1	2	different levels of R	*gyrB* R485H					1	1					
Nosova 2013 &	clinical	1	2	different levels of R	*gyrB* D500H						1	1				
Zimenkov 2013	clinical	1	1	different levels of R	*gyrB* **N538D**							1				
	clinical	1	1		*gyrB* N538S										1	
11) Rigouts	clinical	1	1		*gyrB* T539A				1							
	clinical	1	1		*gyrB* T539N					1						

Notable limitation: Isolates in Study 10 were interrogated using a combination of sequencing and microarray.

7.C.4.3 Conclusion for GFX CC for MGIT

Only two studies were identified that reported GFX MICs for pWT and mutated isolates in MGIT. Owing to the fact that MGIT is widely used, an interim CC of **0.25 mg/L** was, nevertheless, set. Additional data from well-designed studies will be necessary to re-evaluate this CC. As was the case for other media and FQs, *gyrA* mutants can be misclassified as susceptible at this CC due to the overlap between the MIC distributions of pWT and mutated isolates.

7.C.5 References for GFX MIC studies

1. Somasundaram, S. & Paramasivan, N.C. Susceptibility of *Mycobacterium tuberculosis* strains to gatifloxacin and moxifloxacin by different methods. *Chemotherapy* 52, 190-5 (2006).

2. (a) Aung, K.J. *et al.* Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tuberc Lung Dis* 18, 1180-7 (2014).

(b) Rigouts, L. *et al.* Specific *gyrA* gene mutations predict poor treatment outcome in MDR-TB. *J Antimicrob Chemother* 71, 314-23 (2016).

3. Coeck, N. *et al.* Correlation of different phenotypic drug susceptibility testing methods for four fluoroquinolones in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* 71, 1233-40 (2016).

4. Barletta, F., Zamudio, C., Rigouts, L. & Seas, C. Resistance to second-line anti-tuberculosis drugs among Peruvian multidrug resistant *Mycobacterium tuberculosis* strains. *Rev Peru Med Exp Salud Publica* 31, 676-82 (2014).

5. Vincent, V. *et al.* The TDR Tuberculosis Strain Bank: a resource for basic science, tool development and diagnostic services. *Int J Tuberc Lung Dis* 16, 24-31 (2012).

6. Peloquin, C.A. *et al.* Population pharmacokinetics of levofloxacin, gatifloxacin, and moxifloxacin in adults with pulmonary tuberculosis. *Antimicrob Agents Chemother* 52, 852-7 (2008).

7. Rodríguez, J.C., Ruiz, M., López, M. & Royo, G. *In vitro* activity of moxifloxacin, levofloxacin, gatifloxacin and linezolid against *Mycobacterium tuberculosis. Int J Antimicrob Agents* 20, 464-7 (2002).

8. Somasundaram, S. & Paramasivan, N.C. Susceptibility of *Mycobacterium tuberculosis* strains to gatifloxacin and moxifloxacin by different methods. *Chemotherapy* 52, 190-5 (2006).

9. Giannoni, F. *et al.* Evaluation of a new line probe assay for rapid identification of *gyrA* mutations in *Mycobacterium tuberculosis. Antimicrob Agents Chemother* 49, 2928-33 (2005).

10. (a) Isaeva, Y. *et al.* Determination of critical concentrations of moxifloxacin and gatifloxacin

for drug susceptibility testing of *Mycobacterium tuberculosis* in the BACTEC MGIT 960 system. *J Antimicrob Chemother* 68, 2274-81 (2013).

(b) Nosova, E.Y. *et al.* Analysis of mutations in the *gyrA* and *gyrB* genes and their association with the resistance of *Mycobacterium tuberculosis* to levofloxacin, moxifloxacin and gatifloxacin. *J Med Microbiol* 62, 108-13 (2013).

(c) Zimenkov, D.V. *et al.* Detection of second-line drug resistance in *Mycobacterium tuberculosis* using oligonucleotide microarrays. *BMC Infect Dis* 13, 240 (2013).

11. Rigouts, unpublished data.

7.D.1 MFX MIC data on LJ

7.D.1.1 MFX MICs for pWT isolates on LJ

Five studies from two laboratories were identified that reported MFX MIC data for the pWT population on LJ (Table 111). Most distributions were truncated. Somasundaram *et al.* (Study 1) was enriched for OFX-resistant isolates (20 of the 50 isolates tested were resistant to OFX using 2 mg/L as the CC with the absolute concentration method on LJ).

Table 111. MFX MIC distributions for the pWT population on LJ.

						MFX MIC (mg/L)								
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**1**	**2**	**2.5**	**4**	**5**	**8**
1) Somasundaram 2006	1	clinical	50	enriched for OFX R		1	9	**11**	10	6	13			
2) Rigouts 2016	2	clinical	187		gWT		122	49	11	1				4
3) Coeck 2016	2	clinical	45		gWT		15	**19**	5			2	4	
4) Barletta 2014	2	clinical	11	MDR	gWT		5	**5**	1					
5) Vincent 2012	2	clinical	7	different levels of R	gWT		2	**2**		1		2		

Notable limitations: Studies 2-5 were conducted in the same laboratory. Study 1 was enriched for resistant isolates.

7.D.1.2 MFX MICs for mutated isolates on LJ

gyrA mutants

Clinical isolates

67 clinical isolates that were tested in the same laboratory harboured *gyrA* mutations that would be interpreted as conferring resistance using the MTBDR*sl* assays (Table 112). Nine of these mutants (13% (95% CI, 6-24%) had MICs ≤1 mg/L. Eight of these nine isolates (89% (95% CI, 52-100%)) had A90V or D94A mutations.

Table 112. MFX MICs for clinical gyrA mutants on LJ.

						MFX MIC (mg/L)							
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**5**	**8**	**10**
2) Rigouts 2016	2	clinical	1		*gyrA* A74S			1					
2) Rigouts 2016	2	clinical	3		*gyrA* D89N								3
2) Rigouts 2016	2	clinical	13		*gyrA* **A90V**		1	2	10				
3) Coeck 2016	2	clinical	7		*gyrA* **A90V**			1	4	2			
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **A90V**						1		
2) Rigouts 2016	2	clinical	4		*gyrA* **S91P**				2	2			
3) Coeck 2016	2	clinical	2		*gyrA* **S91P**				2				
2) Rigouts 2016	2	clinical	6		*gyrA* **D94A**		1	2	3				
3) Coeck 2016	2	clinical	5		*gyrA* **D94A**			1		3	1		
2) Rigouts 2016	2	clinical	6		*gyrA* **D94G**			1	1	1			3
3) Coeck 2016	2	clinical	14		*gyrA* **D94G**				3	4	7		
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **D94G**						1		
3) Coeck 2016	2	clinical	1		*gyrA* **D94H**						1		
3) Coeck 2016	2	clinical	1		*gyrA* D94N						1		
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* D94N					1			
2) Rigouts 2016	2	clinical	1		*gyrA* **A90V, S91P**								1
5) Vincent 2012	2	clinical	1	different levels of R	*gyrA* **A90V**, D94Y						1		

Notable limitation: All studies were conducted in the same laboratory.

gyrB mutant

Clinical isolate

The only *gyrB* mutant identified, which would have been interpreted as FQ-resistant using the MTBDR*sl* v2 assay, had an MIC of >8 mg/L (Table 113).

Table 113. MFX MICs for clinical gyrB mutants on LJ.

				MFX MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**10**
2) Rigouts 2016	clinical	1	*gyrB* N538S							1

7.D.1.3 Conclusion for MFX CC and CB for LJ

The quantity and quality of data identified for MFX on LJ were similar to GFX on LJ, and consequently the same limitations applied. Taking into account the diagnostic importance of LJ in many settings, an interim CC of **1 mg/L** for LJ was set. It was noted that *gyrA* mutants can be misclassified as susceptible at this concentration due to variations in testing. More MICs for pWT isolates are needed to re-evaluate this CC. A better understanding of the distributions of low- and high-level *gyrA* mutants is needed before a CB can be defined on this medium (Section 7.D.5).

7.D.2 MFX MIC data on 7H10

7.D.2.1 MFX MICs for pWT isolates on 7H10

13 studies from 11 laboratories were identified that reported MFX MIC data for the pWT population on 7H10 (Table 114).

Table 114. MFX MIC distributions for the pWT population on 7H10.

							MFX MIC (mg/L)									
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.02**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
6) Angeby 2010	3		1	4	H37Rv ATCC 27294					**4**						
	3	clinical	114	114	mostly pan-S			4	31	**50**	26	1		1	1	
7) Böttger	4		3	3	H37Rv ATCC 27294					**2**	1					
	4		3	3	Erdman ATCC 35801				1	**2**						
	4	clinical	83	83	pan-S or non-MDR		3	4	**31**	**31**	13	1				
8) de Steenwinkel 2012	5		1	2	H37Rv ATCC 27294						**2**					
	5	clinical	10	20						4	**13**	3				
9) Wang 2007	6	clinical	420	420	different levels of R				20	110	**227**	53	8		1	1
10) Ismail	7	clinical	40	40	different levels of R				4	**12**	**12**	6	1	3	1	1
	7		71	71	different levels of R	gWT			17	**17**	14	11	2	7	1	2
11) Dawson 2015	8	clinical	178	178	pan-S & MDR					108	58	12				
12) Farhat 2015 & 2016	9	clinical	147	147	MDR or XDR	gWT				88	29	20	5		5	
13) Pholwat 2011, 2012	10		1	1	H37Rv ATCC 27294					1						
& 2015	10	clinical	31	31	different levels of R	gWT				27	2	2				
14) van Ingen 2010	11		1	1	H37Rv ATCC 27294					1						
	11	clinical	21	21	MDR	gWT				14	4	3				
15) Diacon 2012	8		1	1	H37Rv ATCC 27294						**1**					
	8	clinical	13	13	mostly pan-S					8	4	1				
16) Peloquin 2008	12		1	1	H37Rv ATCC 27294					1						
	12	clinical	9	9						8	1					
17) Willby 2015	13	clinical	46	46		gWT					35	10	1			
18) Malik 2012	13		1	2	H37Rv						2					
	13		1	2	Erdman						2					
	13		1	2	H37Rv						1	1				
	13		1	2	Erdman						1	1				
	13		1	2	H37Rv						1	1				
	13		1	2	Erdman						1	1				

The green line denotes the current WHO and CLSI CC for MFX DST on 7H10 (0.5 mg/L). The red line denotes the CB recommended by the WHO for MFX DST on 7H10 (2 mg/L). Notable limitations: Studies 11 and 15, and Studies 17 and 18 were conducted in the same laboratory, respectively. In Study 14, the genotypic results were based upon MTBDR*sl* v1 testing.

41 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

Despite truncations in some studies, the modes of five studies could be assessed, including for Ängeby *et al.* (Study 6), which has been cited in the CLSI guidelines in support of the current CC.[41] The modes of the various distributions varied between 0.06 and 0.25 mg/L, and therefore supported the current CC of 0.5 mg/L.

7.D.2.2 MFX MICs for mutated isolates on 7H10

gyrA mutants

Allelic exchange results

Malik *et al.* (Study 18) generated allelic exchange mutants using H37Rv, Erdman and CDC1551 (Table 115). The *gyrA* T80A, G247S, and A384V mutations (or combinations thereof) did not significantly change the MFX MICs (i.e. no MICs for these mutants were above the current MFX CC of 0.5 mg/L). This also applied to the A90G mutation, which has been noted to cause a systematic-false positive result by MTBDRs*l* assays.[42] The MFX MICs of the low-level A90V mutants spanned the CC (with reported MICs of 0.5-1 mg/L), which was also the case for the single A74S mutant. By contrast, the D94G mutants had more highly elevated MFX MICs of 2-16 mg/L.

Table 115. MFX MICs for gyrA allelic exchange mutants on 7H10.

						MFX MIC (mg/L)						
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**
18) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT	2						
		1	2		*gyrA* A74S, **D94G**					1		1
		1	2		*gyrA* T80A	2						
		1	2		*gyrA* T80A, A90G	2						
		1	2		*gyrA* **A90V**		1	1				
		1	2		*gyrA* G247S	2						
		1	2		*gyrA* A384V	2						
		1	2	Erdman	gWT	2						
		1	2		*gyrA* A74S		1	1				
		1	2		*gyrA* T80A	2						
		1	2		*gyrA* T80A, A90G	2						
		1	2		*gyrA* A90G	2						
		1	2		*gyrA* **A90V**		1	1				
		1	2		*gyrA* G247S	2						
		1	2		*gyrA* A384V	2						
		1	2	CDC1551	*gyrA* **D94G**				2			

The green line denotes the current WHO and CLSI CC for MFX DST on 7H10 (0.5 mg/L). The red line denotes the additional WHO CB for MFX DST on 7H10 (2 mg/L).

42 Ajileye, A. *et al.* Some synonymous and nonsynonymous *gyrA* mutations in *Mycobacterium tuberculosis* lead to systematic false-positive fluoroquinolone resistance results with the Hain GenoType MTBDRs*l* assays. *Antimicrob Agents Chemother* 61, e02169-16 (2017)

Clinical isolates

MICs on 7H10 were identified for 177 isolates with *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 116). 23 of these mutants (13% (95% CI, 8-19%)) were phenotypically MFX-susceptible at the current CC. The *gyrA* A90V and D94G mutations accounted for 14 (61% (95% CI, 39-80%)) of these 'susceptible' results.

Table 116. MFX MICs for clinical gyrA mutants on 7H10.

							MFX MIC (mg/L)								
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.06	0.12	0.25	0.5	1	2	2.5	4	8	16
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrA* T80A			2							
17) Willby 2015	13	clinical	1	1		*gyrA* G88C									1	
17) Willby 2015	13	clinical	1	1		*gyrA* D89G					1					
17) Willby 2015	13	clinical	1	1		*gyrA* D89N								1		
12) Farhat 2015 & 2016	9	clinical	3	3	MDR or XDR	*gyrA* D89N					1			2		
10) Ismail	7	clinical	3	3	different levels of R	*gyrA* **A90V**	2	1								
12) Farhat 2015 & 2016	9	clinical	23	23	MDR or XDR	*gyrA* **A90V**			1	3	15			3	1	
14) van Ingen 2010	11	clinical	1	1	MDR	*gyrA* **A90V**							1			
17) Willby 2015	13	clinical	24	24		*gyrA* **A90V**					13	10		1		
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrA* **A90V**					2					
12) Farhat 2015 & 2016	9	clinical	2	2	MDR or XDR	*gyrA* **S91P**					1			1		
14) van Ingen 2010	11	clinical	3	3	MDR	*gyrA* **S91P**						2	1			
17) Willby 2015	13	clinical	1	1		*gyrA* **S91P**						1				
10) Ismail	7	clinical	1	1	different levels of R	*gyrA* **D94A**				1						
12) Farhat 2015 & 2016	9	clinical	12	12	MDR or XDR	*gyrA* **D94A**			2	3	6			1		
17) Willby 2015	13	clinical	12	12		*gyrA* **D94A**				1	7	1		2	1	
10) Ismail	7	clinical	3	3	different levels of R	*gyrA* **D94G**			1	1				1		
12) Farhat 2015 & 2016	9	clinical	26	26	MDR or XDR	*gyrA* **D94G**			2	2	7			15		
13) Pholwat 2011, 2012 & 2015	10	clinical	4	4	different levels of R	*gyrA* **D94G**					2			2		
14) van Ingen 2010	11	clinical	1	1	MDR	*gyrA* **D94G**							1			
17) Willby 2015	13	clinical	25	25		*gyrA* **D94G**				1		2		12	8	2
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrA* **D94G**						2				
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrA* D94N								1		
17) Willby 2015	13	clinical	11	11		*gyrA* D94N								6	4	1
10) Ismail	7	clinical	1	1	different levels of R	*gyrA* D94Y			1							
12) Farhat 2015 & 2016	9	clinical	5	5	MDR or XDR	*gyrA* D94Y					2			1	2	
13) Pholwat 2011, 2012 & 2015	10	clinical	1	1	different levels of R	*gyrA* D94Y				1						
17) Willby 2015	13	clinical	2	2		*gyrA* D94Y					1	1				
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrA* G247S			2							
17) Willby 2015	13	clinical	1	1		*gyrA* **A90V, S91P**						1				
14) van Ingen 2010	11	clinical	1	1	MDR	*gyrA* **A90V, D94A**						1				
17) Willby 2015	13	clinical	1	1		*gyrA* **A90V, D94G**								1		
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrA* **S91P, D94G**								1		
17) Willby 2015	13	clinical	1	1		*gyrA* **S91P, D94G**								1		
17) Willby 2015	13	clinical	1	1		*gyrA* G88C, **A90V, D94G**									1	
14) van Ingen 2010	11	clinical	1	1	MDR	*gyrA* **A90V, S91P**, D94N or D94Y							1			

The green line denotes the current WHO and CLSI CC for MFX DST on 7H10 (0.5 mg/L). The red line denotes the additional WHO CB for MFX DST on 7H10 (2 mg/L). Notable limitations: Studies 17 and 18 were conducted in the same laboratory. In Study 14, the genotypic results were based upon MTBDR*sl* v1 testing.

gyrB mutants

Allelic exchange results

Malik *et al.* (Study 18) generated allelic exchange mutants using H37Rv and Erdman (Table 117). The MFX MICs of some of the *gyrB* mutants were elevated above the current MFX CC (e.g. N538K mutants had MICs 1-2 mg/L in both genetic backgrounds tested). However, the majority of MFX MICs were ≤0.25-0.5 mg/L, and were consequently susceptible based on the current MFX CC of 0.5 mg/L, as were the gWT control strains (MICs of ≤0.25 mg/L). These isolates included several mutants that would be interpreted as resistant by the MTBDRs*l* v2 assay (e.g. T539N). However, the MFX MICs for several of these 'LPA-resistant' isolates were equal to the CC, suggesting that these mutations may only result in subtle MIC increases.

Table 117. MFX MICs for gyrB allelic exchange mutants on 7H10.

						MFX MIC (mg/L)				
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**
18) Malik 2012	allelic exchange mutants	1	2	H37Rv	gWT	1	1			
		1	2		*gyrB* V340L	2				
		1	2		*gyrB* R485C	2				
		1	2		*gyrB* R485C, T539N				2	
		1	2		*gyrB* D500A	1	1			
		1	2		*gyrB* D500H	1	1			
		1	2		*gyrB* D500N	1	1			
		1	2		*gyrB* D533A	2				
		1	2		*gyrB* **N538D**			2		
		1	2		*gyrB* **N538D**, T546M			2		
		1	2		*gyrB* N538K			1	1	
		1	2		*gyrB* N538T, T546M		1	1		
		1	2		*gyrB* T539N	1	1			
		1	2		*gyrB* T539P		1	1		
		1	2		*gyrB* E540D				1	1
		1	2		*gyrB* **E540V**		1	1		
		1	2		*gyrB* A543T	1	1			
		1	2		*gyrB* A543V		2			
		1	2		*gyrB* T546M	2				
		1	2	Erdman	gWT	1	1			
		1	2		*gyrB* M330I	2				
		1	2		*gyrB* V340L	2				
		1	2		*gyrB* R485C	1	1			
		1	2		*gyrB* R485C, T539N					2
		1	2		*gyrB* D500A	1	1			
		1	2		*gyrB* D500H		2			
		1	2		*gyrB* D500N		2			
		1	2		*gyrB* D533A	2				
		1	2		*gyrB* **N538D**			2		
		1	2		*gyrB* **N538D**, T546M			1	1	
		1	2		*gyrB* N538K			1	1	
		1	2		*gyrB* N538T, T546M	1		1		
		1	2		*gyrB* T539N			2		
		1	2		*gyrB* T539P		1	1		
		1	2		*gyrB* E540D				2	
		1	2		*gyrB* **E540V**			2		
		1	2		*gyrB* A543T	1	1			
		1	2		*gyrB* A543V		1	1		
		1	2		*gyrB* T546M	2				

The green line denotes the current WHO and CLSI CC for MFX DST on 7H10 (0.5 mg/L). The red line denotes the additional WHO CB for MFX DST on 7H10 (2 mg/L).

Clinical isolates

Excluding the *gyrB* V340L mutation, which does not cause FQ resistance, 16 clinical isolates from four studies harboured *gyrB* mutations (Table 118). These included nine isolates that would be considered resistant by the MTBDR*sl* v2 assay, of which two were susceptible at the CC (22%, 95% CI, 3-60%).

Table 118. MFX MICs for clinical gyrB mutants on 7H10.

							MFX MIC (mg/L)						
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* V340L		2					
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrB* R485H				1			
13) Pholwat 2011, 2012 & 2015	10	clinical	1	1	different levels of R	*gyrB* S486F			1				
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrB* S486Y				1			
17) Willby 2015	13	clinical	1	1		*gyrB* D500H				1			
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* D500H				2			
17) Willby 2015	13	clinical	2	2		*gyrB* D500N			2				
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrB* **N538D**						1	
17) Willby 2015	13	clinical	1	1		*gyrB* **N538D**					1		
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* **N538D**				2			
17) Willby 2015	13	clinical	1	1		*gyrB* N538K						1	
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrB* T539A		1					
12) Farhat 2015 & 2016	9	clinical	1	1	MDR or XDR	*gyrB* E540D						1	
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* R485C, T539N					1	1	
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* **N538D**, T546M				2			
18) Malik 2012	13	clinical	1	2	different levels of R	*gyrB* N538T, T546M		2					

The green line denotes the current WHO and CLSI CC for MFX DST on 7H10 (0.5 mg/L). The red line denotes the additional WHO CB for MFX DST on 7H10 (2 mg/L). Notable limitation: Studies 17 and 18 were conducted in the same laboratory.

7.D.2.3 Conclusion for MFX CC and CB for 7H10

Based upon the identified MIC data, the current MFX CC of **0.5 mg/L** was reaffirmed, although there was some overlap between the pWT MIC distribution and *gyrA* mutants, which meant that some mutants were misclassified as susceptible.

The CB was designed to distinguish low-level *gyrA* mutants (e.g. A90V and D94A) from high-level mutants (the rationale for this decision can be found in Section 7.D.5). However, it should also be noted that the lower end of the MIC distribution of high-level resistance mutations (e.g. *gyrA* D94G) appears to be **2 mg/L**, meaning that a proportion of these mutants will be misclassified as low-level resistant using the current CB due to the inherent variation in testing. This could be avoided by lowering the CB to 1 mg/L at the expense of increasing substantially the proportion of low-level resistant isolates that are misclassified as high-level resistant. Faced with this trade-off, 2 mg/L was reaffirmed as the CB. However, it was suggested that in order to minimise misclassifying high-level resistant strains as low-level resistant, the identification of high-level resistance mutations using a molecular assay (e.g. LPA identification of a *gyrA* D94G mutation) might overrule a susceptible phenotypic DST result at 2 mg/L. This proposal will be addressed in a later report.

7.D.3 MFX MIC data on 7H11

7.D.3.1 MFX MICs for pWT isolates on 7H11

Five studies were identified that reported MFX MIC data for the pWT population on 7H11 (Table 119). This included Somasundaram *et al.* (Study 22), which has been cited in the CLSI guidelines in support of a CC.[43] This study was enriched for OFX-resistant isolates (20 of the 50 isolates tested were resistant to OFX using 2 mg/L as the CC with the absolute concentration method on LJ). Given the modes of these MIC distributions, which varied between 0.12-0.5 mg/L, the current CLSI CC of 0.5 mg/L appeared to be appropriate.

Table 119. MFX MICs for pWT isolates on 7H11.

						MFX MIC (mg/L)								
Studies	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
19) Giannoni 2005 & Piersimoni 2007	clinical	20	20	H37Rv ATCC 27294 & pan-S			2	**15**	1	2				
		10	10	MDR			2	**6**	1	1				
		10	10	MDR	gWT			**5**		1	2	1	1	
20) Rodríguez 2002	clinical	243	243	mostly pan-S			7	27	**174**	29	1	2	1	2
21) López-Gavín 2015	clinical	11	11	pan-S				**11**						
		7	7	MDR				**7**						
22) Somasundaram 2006	clinical	50	50	enriched for OFX R				2	10	**17**	5			
23) Bernard 2016		1	6	H37Rv	parent strain				3	3				

The blue line denotes the current CLSI CC for MFX DST on 7H11 (0.5 mg/L). Notable limitation: Study 22 was enriched for resistant isolates.

7.D.3.2 MFX MICs for mutated isolates on 7H11

gyrA mutants

Clinical isolates

44 isolates from two studies were identified with *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 120). Only one mutant (2% (95% CI, 0-12%)) was susceptible at the CLSI CC.

Table 120. MFX MICs for mouse and clinical gyrA mutants on 7H11.

					MFX MIC (mg/L)					
Studies	**Isolate origin**	**Unique isolates**	**Type of isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
23) Bernard 2016	mice	2		*gyrA* D89G	1		1			
23) Bernard 2016	mice	6		*gyrA* D89N					3	3
19) Giannoni 2005 & Piersimoni 2007	clinical	9	MDR	*gyrA* **A90V**			6	2	1	
23) Bernard 2016	mice	2		*gyrA* **A90V**				1	1	
19) Giannoni 2005 & Piersimoni 2007	clinical	2	MDR	*gyrA* **S91P**			1	1		
23) Bernard 2016	mice	2		*gyrA* **S91P**					2	
19) Giannoni 2005 & Piersimoni 2007	clinical	1	MDR	*gyrA* **D94A**			1			
23) Bernard 2016	mice	1		*gyrA* **D94A**				1		
19) Giannoni 2005 & Piersimoni 2007	clinical	4	MDR	*gyrA* **D94G**			2	1	1	
23) Bernard 2016	mice	7		*gyrA* **D94G**					5	2
23) Bernard 2016	mice	2		*gyrA* **D94H**					1	1
19) Giannoni 2005 & Piersimoni 2007	clinical	1	MDR	*gyrA* D94N			1			
23) Bernard 2016	mice	2		*gyrA* D94N						2
23) Bernard 2016	mice	3		*gyrA* D94Y				1		2

The blue line denotes the current CLSI CC for MFX DST on 7H11 (0.5 mg/L).

43 Clinical and Laboratory Standards Institute. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 2nd edition Approved standard. CLSI document M24-A2. (2011).

gyrB mutants

Clinical isolates

12 *gyrB* mutants that arose during the treatment of mice with LFX were tested on 7H11, of which two had mutations targeted by the MTBDRsl v2 assay (Table 121). One of these was MFX-susceptible at the current CC (50% (95% CI, 1-99%)).

Table 121. MFX MICs for mouse gyrB mutants on 7H11.

				MFX MIC (mg/L)				
Studies	**Isolate origin**	**Unique isolates**	**Genotypic results**	**0.25**	**0.5**	**1**	**2**	**4**
23) Bernard 2016	mice	1	*gyrB* R485C		1			
		1	*gyrB* D500A			1		
		4	*gyrB* D500H				4	
		2	*gyrB* D500N			2		
		1	*gyrB* N538T				1	
		1	*gyrB* E540A		1			
		2	*gyrB* A543V		1	1		

The blue line denotes the CLSI CC for MFX DST on 7H11 media.

7.D.3.3 Conclusion for MFX CC and CB for 7H11

Based on the data from the five studies identified in this review, **0.5 mg/L** was adopted as the CC, but a better understanding of the distributions of low- and high-level *gyrA* mutants was needed before a CB could be defined on this medium (Section 7.D.5).

7.D.4 MFX MIC data in MGIT

7.D.4.1 MFX MICs for pWT isolates in MGIT

16 studies were identified that reported MFX MIC data for the pWT population in MGIT (Table 122). Despite the truncation of most MIC distributions, the CLSI CC of 0.25 mg/L, rather than the current WHO CC of 0.5 mg/L, appeared to correspond to the upper end of the pWT MIC distribution.

Table 122. MFX MICs for pWT isolates in MGIT.

							MFX MIC (mg/L)										
Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.06	0.12	0.18	0.25	0.5	0.75	1	1.5	2	2.5	4	8
24) Isaeva 2013,	18		1	1	H37Rv ATCC 25618			**1**										
Nosova 2013 &	18	clinical	11	11	different levels of R		1	**9**		1								
24) Isaeva 2013,	18	clinical	23	23	different levels of R	gWT	1	**18**		4								
25) Kambli 2015	19		1	1	H37Rv ATCC 27294		1											
	19	clinical	30	30		gWT	26	2		2								
26) Piersimoni 2007	20		20	20	H37Rv ATCC 27294 & pan-S		5	**11**		3	1							
	20	clinical	10	10			2	**5**		2			1					
	20		1	1		gWT									1			
27) Tessema 2017	21	clinical	41	41		gWT	8	**32**		1								
28) Heyckendorf	21		1	1	H37Rv ATCC 27294					**1**								
2017	21	clinical	16	16	MDR or XDR	gWT	9	7										
29) Sirgel 2012	22		1	6	H37Rv ATCC 27294			6										
	22	clinical	125	125	different levels of R	gWT		119		5					1			
30) Krüüner 2006	23		132	132	MDR			97			5		16	14				
31) van Ingen 2010	11		1	1	H37Rv ATCC 27294			1										
	11	clinical	21	21	MDR	gWT		20		1								
32) Rigouts	2		1	5	H37Rv ATCC 27294			4	1									
	2	clinical	9	9		gWT		9										
33) Ismail	7	clinical	57	57	different levels of R			26		12	2		2		12		2	1
	7		3	3	different levels of R	gWT		2					1					
34) Lin	24	clinical	73	73		gWT		44		22	1	1	1	3	1			
	24		218	218		gWT				215			2		1			
35) Cambau 2015	4, 11, 21, 25-31	clinical	114	114	MDR					114								
	4, 11, 21, 25-31		3	3	MDR	gWT				3								
36) Sharma 2011	32-34	clinical	36	144	different levels of R					101	6		12	25				
37) Alvarez 2014	35		1	1	H37Rv					1								
	35	clinical	5	5	MDR or XDR	gWT				1	1		2		1			
38 Kam 2006	36	clinical	108	108	mostly MDR						108							
	36		4	4	mostly MDR	gWT							2		2			
39) Rodrigues 2008	19	clinical	1	1	H37Rv ATCC 27294								1					
	19		10	10	pan-S								10					
	19		20	20	different levels of R								19		1			

The blue line denotes the current CLSI CC for MFX DST in MGIT (0.25 mg/L). The red lines denote the current WHO CC and CB for MFX DST in MGIT (0.5 and 2 mg/L). Notable limitations: Studies 25 and 39 were conducted in the same laboratory and some laboratories were in common to Studies 27, 28, 31 and 35. In Study 24, the genotypic results were based upon a combination of sequencing and microarray, whereas Study 25 used the MTBDR*sl* v1.

7.D.4.2 MFX MICs for mutated isolates in MGIT

gyrA mutants

Clinical isolates

416 isolates from 12 studies had *gyrA* mutations that are targeted by the MTBDR*sl* assays (Table 123). Using the CLSI CC of 0.25 mg/L for MGIT, only 19 (5% (95% CI, 3-7%)) of these *gyrA* mutants were phenotypically MFX-susceptible. Notably, 100% (95% CI, 82-100%) of these 'susceptible' results, which were likely due to variation in testing, were for isolates with A90V and D94A mutations.

Table 123. MFX MICs for clinical gyrA mutants in MGIT.

							MFX MIC (mg/L)														
Studies	**Lab**	**Isolate origin**	**Unique isolates**	**Total MICs**	**Type of isolates**	**Genotypic results**	**0.12**	**0.18**	**0.25**	**0.5**	**0.75**	**1**	**1.5**	**2**	**2.5**	**3**	**4**	**5**	**7.5**	**8**	**10**
29) Sirgel 2012	22	clinical	1	1	different levels of R	*gyrA* T80S	1														
24) Isaeva 2013, Nosova	18	clinical	2	2	different levels of R	*gyrA* G88A						1		1							
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	*gyrA* G88C											1				
29) Sirgel 2012	22	clinical	1	1	different levels of R	*gyrA* G88C											1				
34) Lin	24	clinical	1	1		*gyrA* G88C											1				
29) Sirgel 2012	22	clinical	1	1	different levels of R	*gyrA* D89G						1									
27) Tessema 2017	21	clinical	1	1		*gyrA* D89N									1						
32) Rigouts	2	clinical	1	1		*gyrA* D89N				1											
35) Cambau 2015	4, 11, 21, 25-31	clinical	1	1	MDR	*gyrA* D89N				1											
34) Lin	24	clinical	1	1		*gyrA* A90K						1									
24) Isaeva 2013, Nosova	18	clinical	10	10	different levels of R	***gyrA* A90V**				5		2		3							
25) Kambli 2015	19	clinical	25	25		***gyrA* A90V**			5	4		16									
26) Piersimoni 2007	20	clinical	5	5		***gyrA* A90V**						4		1							
29) Sirgel 2012	22	clinical	12	12	different levels of R	***gyrA* A90V**			4			8									
31) van Ingen 2010	11	clinical	1	1	MDR	***gyrA* A90V**							1								
32) Rigouts	2	clinical	20	20		***gyrA* A90V**		1	6	8		5									
33) Ismail	7	clinical	2	2	different levels of R	***gyrA* A90V**						1		1							
34) Lin	24	clinical	15	15		***gyrA* A90V**				5	7	3									
34) Lin	24	clinical	8	8		***gyrA* A90V**				1		7									
35) Cambau 2015	4, 11, 21, 25-31	clinical	3	3	MDR	***gyrA* A90V**				2					1						
37) Alvarez 2014	35	clinical	2	2	MDR or XDR	***gyrA* A90V**				2											
38 Kam 2006	36	clinical	8	8	mostly MDR	***gyrA* A90V**				2		5		1							
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	***gyrA* S91P**						1									
25) Kambli 2015	19	clinical	6	6		***gyrA* S91P**						6									
26) Piersimoni 2007	20	clinical	2	2		***gyrA* S91P**								2							
29) Sirgel 2012	22	clinical	3	3	different levels of R	***gyrA* S91P**				1		2									
31) van Ingen 2010	11	clinical	3	3	MDR	***gyrA* S91P**						1	2								
32) Rigouts	2	clinical	5	5		***gyrA* S91P**				1		4									
34) Lin	24	clinical	2	2		***gyrA* S91P**						1	1								
34) Lin	24	clinical	8	8		***gyrA* S91P**						4		4							
35) Cambau 2015	4, 11, 21, 25-31	clinical	5	5	MDR	***gyrA* S91P**									4					1	
37) Alvarez 2014	35	clinical	1	1	MDR or XDR	***gyrA* S91P**											1				
38 Kam 2006	36	clinical	1	1	mostly MDR	***gyrA* S91P**						1									
24) Isaeva 2013, Nosova	18	clinical	6	7	different levels of R	***gyrA* D94A**				2		4		1							
25) Kambli 2015	19	clinical	4	4		***gyrA* D94A**						1			3						
29) Sirgel 2012	22	clinical	7	7	different levels of R	***gyrA* D94A**						4		3							
32) Rigouts	2	clinical	11	11		***gyrA* D94A**			3	5		3									
33) Ismail	7	clinical	1	1	different levels of R	***gyrA* D94A**				1											
34) Lin	24	clinical	6	6		***gyrA* D94A**				1	3	1	1								
34) Lin	24	clinical	3	3		***gyrA* D94A**				1		2									
35) Cambau 2015	4, 11, 21, 25-31	clinical	2	2	MDR	***gyrA* D94A**									2						
38 Kam 2006	36	clinical	5	5	mostly MDR	***gyrA* D94A**				1		4									
34) Lin	24	clinical	1	1		*gyrA* D94F						1									
24) Isaeva 2013, Nosova	18	clinical	13	13	different levels of R	***gyrA* D94G**								13							
25) Kambli 2015	19	clinical	42	42		***gyrA* D94G**						9			32					1	
26) Piersimoni 2007	20	clinical	2	2		***gyrA* D94G**								2							
29) Sirgel 2012	22	clinical	17	17	different levels of R	***gyrA* D94G**						3		14							
31) van Ingen 2010	11	clinical	1	1	MDR	***gyrA* D94G**							1								
32) Rigouts	2	clinical	20	20		***gyrA* D94G**				1		9	10								
33) Ismail	7	clinical	2	2	different levels of R	***gyrA* D94G**								1			1				
34) Lin	24	clinical	15	15		***gyrA* D94G**						2	5	4		2	2				
34) Lin	24	clinical	16	16		***gyrA* D94G**						1		13		1	1				
35) Cambau 2015	4, 11, 21, 25-31	clinical	7	7	MDR	***gyrA* D94G**									6				1		
37) Alvarez 2014	35	clinical	4	4	MDR or XDR	***gyrA* D94G**						2		2							
38 Kam 2006	36	clinical	12	12	mostly MDR	***gyrA* D94G**								11			1				
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	***gyrA* D94H**								1							
25) Kambli 2015	19	clinical	3	3		***gyrA* D94H**						1			2						
32) Rigouts	2	clinical	1	1		***gyrA* D94H**								1							
34) Lin	24	clinical	4	4		***gyrA* D94H**							2	2							
35) Cambau 2015	4, 11, 21, 25-31	clinical	1	1	MDR	***gyrA* D94H**									1						
38 Kam 2006	36	clinical	1	1	mostly MDR	***gyrA* D94H**								1							
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	*gyrA* D94N											1				
29) Sirgel 2012	22	clinical	6	6	different levels of R	*gyrA* D94N				1				4			1				
32) Rigouts	2	clinical	1	1		*gyrA* D94N									1						
33) Ismail	7	clinical	2	2	different levels of R	*gyrA* D94N						1		1							
34) Lin	24	clinical	1	1		*gyrA* D94N											1				
34) Lin	24	clinical	6	6		*gyrA* D94N								2		2	2				
35) Cambau 2015	4, 11, 21, 25-31	clinical	1	1	MDR	*gyrA* D94N									1						
25) Kambli 2015	19	clinical	13	13		*gyrA* D94N or D94Y									11			2			
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	*gyrA* D94Y						1									
32) Rigouts	2	clinical	1	1		*gyrA* D94Y								1							
33) Ismail	7	clinical	1	1	different levels of R	*gyrA* D94Y								1							
34) Lin	24	clinical	1	1		*gyrA* D94Y								1							
34) Lin	24	clinical	1	1		*gyrA* D94Y								1							
38 Kam 2006	36	clinical	2	2	mostly MDR	*gyrA* D94Y								1			1				
31) van Ingen 2010	11	clinical	1	1	MDR	***gyrA* A90V, D94A**							1								
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	***gyrA* A90V, D94G**															1
29) Sirgel 2012	22	clinical	2	2	different levels of R	***gyrA* A90V, D94G**						1		1							
35) Cambau 2015	4, 11, 21, 25-31	clinical	1	1	MDR	***gyrA* A90V, D94G**														1	
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	***gyrA* A90V**, D94Y						1									
38 Kam 2006	36	clinical	2	2	mostly MDR	***gyrA* A90V**, P102H				1		1									
24) Isaeva 2013, Nosova	18	clinical	1	1	different levels of R	***gyrA* S91P**, D94N								1							
29) Sirgel 2012	22	clinical	2	2	different levels of R	***gyrA* D94G**, D94N								2							
31) van Ingen 2010	11	clinical	1	1	MDR	***gyrA* A90V, S91P**, D94N or D94Y							1								

The blue line denotes the current CLSI CC for MFX DST in MGIT (0.25 mg/L). The red lines denote the current WHO CC and CB for MFX DST in MGIT (0.5 and 2 mg/L). Notable limitations: Some laboratories were in common to Studies 27, 31 and 35. In Study 24, the genotypic results were based upon a combination of sequencing and microarray, whereas Study 25 used the MTBDRs/ v1.

gyrB mutants

Clinical isolates

13 isolates from three laboratories had *gyrB* mutations (Table 124). These included five isolates that would be considered resistant by the MTBDRs*l* v2 assay, of which one (20% (95% CI, 1-72%)) was susceptible at 0.25 mg/L.

Table 124. MFX MICs for clinical gyrB mutants in MGIT.

						MFX MIC (mg/L)										
Studies	Lab	Isolate origin	Unique isolates	Type of isolates	Genotypic results	0.06	0.12	0.18	0.25	0.5	1	1.5	2	2.5	4	7.5
24) Isaeva 2013, Nosova	18	clinical	1	different levels of R	*gyrB* R485H				1							
27) Tessema 2017	21	clinical	3		*gyrB* R485H				3							
27) Tessema 2017	21	clinical	1		*gyrB* S486Y					1						
24) Isaeva 2013, Nosova	18	clinical	1	different levels of R	*gyrB* D500H					1						
24) Isaeva 2013, Nosova	18	clinical	1	different levels of R	*gyrB* **N538D**					1						
32) Rigouts	2	clinical	1		*gyrB* N538S							1				
32) Rigouts	2	clinical	1		*gyrB* T539A					1						
32) Rigouts	2	clinical	1		*gyrB* T539N				1							
27) Tessema 2017	21	clinical	1		*gyrB* E540D									1		
28) Heyckendorf 2017	21	clinical	2	MDR or XDR	*gyrB* A543V		1		1							

The blue line denotes the current CLSI CC for MFX DST in MGIT (0.25 mg/L). The red lines denote the current WHO CC and CB for MFX DST in MGIT (0.5 and 2 mg/L). Notable limitations: Studies 27 and 28 were conducted in the same laboratory. In Study 24, the genotypic results were based upon a combination of sequencing and microarray.

7.D.4.3 Conclusion for MFX CC and CB for MGIT

The CC was lowered from 0.5 to **0.25 mg/L** for MGIT. There was some overlap between the MIC distributions of low-level *gyrA* mutants and pWT isolates, resulting in false-susceptible results. The CB was also lowered from 2 to **1 mg/L**, as this captured most low-level resistant mutants (e.g. *gyrA* A90V and D94A). As was the case on 7H10, the distributions of low-level and high-level mutants overlapped, which meant that some high-level mutants were misclassified even at the lower CB (the rationale for the CB can be found in Section 7.D.5).

7.D.5 Rationale for MFX CB

This CC is relevant for the daily dose 400 mg of MFX. One study conducted by Rigouts *et al.* showed that despite the higher MIC values for non-wild type isolates the outcome was still better with a high dose of GFX than without a FQ being included in the regimen.[44] This is likely caused by synergy between GFX and other drug compounds in the regimen. Therefore, given treatment with a combination regimen, a high dose of MFX is of additional value (extrapolated from treatment with a high dose of the GFX). At MIC values >1 mg/L of GFX on LJ, however, the success rate dropped dramatically.[44, 45]

Of note, however, the CBs (1 mg/L in MGIT and 2 mg/L in 7H10) are for a daily dose of 800 mg of MFX.

For any strain that is MFX-resistant at the CB of 1 mg/L in MGIT or 2 mg/L in 7H10 (i.e. with MICs above these values), MFX should not be considered as an effective drug in the regimen. Strains that have elevated MICs above the CC but below or equal to the CB may be effectively treated with the higher daily dose of 800 mg MFX. Strains with MICs at or below the CC are likely to be effectively treated at a lower daily dose of MFX of 400 mg.

44 Rigouts, L. *et al.* Specific *gyrA* gene mutations predict poor treatment outcome in MDR-TB. *J Antimicrob Chemother* 71, 314-23 (2016).
45 Jan-Willem Alffenaar, Tawanda Gambo, personal communication (September 2017)

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