# Supplement

**Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis [WHO/CDS/TB/2018.5]. Available from: http://apps.who.int/iris/bitstream/10665/260470/1/WHO-CDS-TB-2018.5-eng.pdf Geneva: World Health Organization; 2018.**

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## Conflicts of interest

C. U. K. is a research associate at Wolfson College and visiting scientist at the Department of Genetics, University of Cambridge. He has collaborated with Illumina Inc. on a number of scientific projects and is a consultant for the Foundation for Innovative New Diagnostics. The Bill & Melinda Gates Foundation, Janssen Pharmaceutica, and PerkinElmer covered C. U. K.'s travel and accommodation to present at meetings. The European Society of Mycobacteriology awarded C. U. K. the Gertrud Meissner Award, which is sponsored by Hain Lifescience.

T. S. is the scientific secretary of the EUCAST subgroup on antimycobacterial susceptibility testing and the ESCMID study group for mycobacterial infections.

B. V. none to declare.

S. G. is a Scientific Officer at FIND, a not-for-profit foundation, whose mission is to find diagnostic solutions to overcome diseases of poverty in LMICs. It works closely with the private and public sectors and receives funding from some of its industry partners. It has organizational firewalls to protect it against any undue influences in its work or the publication of its findings. All industry partnerships are subject to review by an independent Scientific Advisory Committee or another independent review body, based on due diligence, TPPs and public sector requirements. FIND catalyses product development, leads evaluations, takes positions, and accelerates access to tools identified as serving its mission. It provides indirect support to industry partners (e.g. access to open specimen banks, a clinical trial platform, technical support, expertise, laboratory capacity strengthening in LMICs, etc.) to facilitate the development and use of products in these areas. FIND also supports the evaluation of publicly-prioritized TB assays and the implementation of WHO-approved (guidance & PQ) assays using donor grants. In order to carry out test evaluations, FIND has product evaluation agreements with several private sector companies for TB and other diseases, which strictly define its independence and neutrality vis-a-vis the companies whose products get evaluated and describes roles and responsibilities.

## Acknowledgments

We would like to thank our colleagues at FIND for enabling this work. We are particularly indebted to David Dolinger for the conception and initiation of this project, as well as his guidance and input on early versions of this report. We would also like to acknowledge the hundreds of researchers from across the globe who have contributed to this review by sharing raw data or clarifying experimental details not included in their publications. Finally, we are grateful to Sheila Palmer (Medical Library, University of Cambridge) as well as Rasha Jerandi and Clare Pierard (both International Journal of Tuberculosis and Lung Disease) for supplying literature that was not available electronically.

## Reporting of additional MIC data and any identified errors

If you identify any errors in the report, or if you have any additional MIC data to contribute to this review, particularly for antibiotics for which no critical concentrations could be defined, please contact Sophia Georghiou (sophia.georghiou@finddx.org) to facilitate the timely re-evaluation of the conclusions in this report.

# 1 Excel MIC datasheets

The detailed MIC data for all studies included in the WHO report can be found in the accompanying Excel datasheets, which can be filtered by specific identifiers (e.g. 'dataset' or 'filter numbers') to highlight particular MIC data. For example, filter K1 would display the KAN MICs for pWT isolates on LJ (i.e. Table 4 in the main report). This filter may be applied by opening the 'KAN' worksheet in the 'SLI MICs v 7_2' file and filtering column G ('filter') to display only those records with '1' entered in this column. For these datasheets, it should be noted that the Excel filter function may only be used to filter columns with entries that have **not** been merged (e.g. it is possible to filter by 'medium' but not by the PMID, since the latter would only select the first row with the corresponding PMID). Table 1 lists the corresponding MIC data filters for each MIC table featured in the WHO report.

**Table 1.** List of MIC data filters corresponding to each MIC table in WHO report.

| Table number | Corresponding Excel MIC data |
|---|---|
| 4 | filter K1 |
| 5 | filter K2 |
| 6 | filter K3 |
| 7 | dataset K11 |
| 8 | filter K4 |
| 9 | datasets K12 & K17 |
| 10 | filter K5 |
| 11 | datasets K14-16 |
| 12 | filter K6 |
| 13 | filter K7 |
| 14 | filter K8 |
| 15 | filter K9 |
| 16 | filter K10 |
| 17 | filter K11 |
| 18 | filter A1 |
| 19 | filter A2 |
| 20 | filter A3 |
| 21 | dataset A12 |
| 22 | filter A4 |
| 23 | datasets A13 & A16 |
| 24 | filter A5 |
| 25 | filter A6 |

| | |
|---|---|
| 26 | filter A7 |
| 27 | filter A8 |
| 28 | filter A9 |
| 29 | filter A10 |
| 30 | filter A11 |
| 31 | filter A12 |
| 32 | filter A13 |
| 33 | filter P1 |
| 34 | filter P2 |
| 35 | filter P3 |
| 36 | dataset P10 |
| 37 | filter P4 |
| 38 | filter P5 |
| 39 | filter P6 |
| 40 | filter P7 |
| 41 | filter P8 |
| 42 | filter P9 |
| 43 | filter P10 |
| 44 | filter P11 |
| 45 | filter P12 |
| 46 | filter P13 |
| 47 | filter P14 |
| 48 | filter C1 |
| 49 | filter C2 |
| 50 | filter C3 |
| 51 | filter C4 |
| 52 | filter B1 |
| 53 | filter B2 |
| 54 | filter B3 |
| 55 | filter B4 |
| 56 | filter B5 |
| 58 | filter S1 |
| 59 | filter S2 |
| 60 | filter S3 |

| | |
|---|---|
| 61 | filter S4 |
| 63 | filter Z1 |
| 64 | filter Z2 |
| 65 | filter Z3 |
| 66 | dataset Z25 |
| 67 | filter Z4 |
| 68 | filter D1 |
| 69 | filter D2 |
| 70 | filter D3 |
| 71 | filter D4 |
| 72 | filter D5 |
| 74 | filter O1 |
| 75 | filter O2 |
| 76 | filter O3 |
| 77 | filter O4 |
| 78 | dataset O13 |
| 79 | filter O5 |
| 80 | dataset O14 |
| 81 | filter O6 |
| 82 | filter O7 |
| 83 | filter O8 |
| 84 | filter O9 |
| 85 | filter O10 |
| 86 | filter O11 |
| 87 | filter O12 |
| 88 | filter L1 |
| 89 | filter L2 |
| 90 | filter L3 |
| 91 | filter L4 |
| 92 | dataset L8 |
| 93 | filter L5 |
| 94 | dataset L9 |
| 95 | filter L6 |
| 96 | filter L7 |

| | |
|---|---|
| 97 | filter L8 |
| 98 | filter L9 |
| 99 | filter L10 |
| 100 | filter L11 |
| 101 | filter L12 |
| 102 | filter G1 |
| 103 | filter G2 |
| 104 | filter G3 |
| 105 | filter G4 |
| 106 | filter G5 |
| 107 | filter G6 |
| 108 | filter G7 |
| 109 | filter G8 |
| 110 | filter G9 |
| 111 | filter M1 |
| 112 | filter M2 |
| 113 | filter M3 |
| 114 | filter M4 |
| 115 | dataset M18 |
| 116 | filter M5 |
| 117 | dataset M19 |
| 118 | filter M6 |
| 119 | filter M7 |
| 120 | filter M8 |
| 121 | filter M9 |
| 122 | filter M10 |
| 123 | filter M11 |
| 124 | filter M12 |

The following one-letter abbreviations are used for the respective worksheets: A=AMK, B=BDQ, C=CFZ, D=DLM, G=GFX, K=KAN, L=LFX, M=MFX, O=OFX, P=CAP, S=DCS, and Z=LZD

# 2 Sources of bias

## 2.1 Sampling

The only piece of information that could be included for all studies was the country in which MIC testing was done, which did not necessarily correspond to the country of origin of the tested isolates. Each laboratory was assigned a 'lab' number in the Excel datasheets. The corresponding lab numbers of sets of isolates that were tested in the same laboratory (using the same medium) were highlighted in red to indicate that these datasets should not be regarded as independent.

The year of isolation was only available for some studies and the vast majority of studies did not include any phylogenetic information (i.e. it could not be assessed what proportion of the MTBC diversity was sampled, nor whether isolates were part of an outbreak, in which case the same strain was likely sampled repeatedly).[1] The studies that did report typing results used a wide variety of methods. Phylogenetic data were therefore not included in this review, although a few exceptions were made for important drug resistance mechanisms (e.g. typing results were discussed in the DLM chapter for isolates that were naturally resistant to DLM[2]).

## 2.2 Data stratification

When possible, MIC data were further stratified based on the drug resistance profile of the given isolates, as susceptible strains are more likely to be pWT for any drug, and were consequently more suitable to define a CC than strains showing any drug resistance. Conversely, MDR and XDR strains are generally more likely to have been exposed to second-line TB drugs and were consequently more likely to be pNWT.

MIC data were stratified by genotypic DST results whenever available. The resistance gene(s) interrogated in each study were listed in the 'comment' column of the Excel datasheets. In the vast majority of cases, these results were based on sequencing. For a limited number of studies with notable MIC results (e.g. datasets for which a broad range of concentrations were tested), data were also stratified based on genotypic data that were obtained using other methods (e.g. using the Hain Genotype MTBDR*sl* assay[3] or a through a combination of both sequencing and microarray data[4]). These studies have been clearly marked in the 'comment' column of the Excel datasheets.

It should be noted that some studies only sequenced resistant isolates (e.g. Cambau *et al.*[5]), which could result in an overestimation of the specificity of sequencing given the inclusion of data from these studies. The 'genotypic results' entry was left blank in these cases (i.e. only where sequencing was performed and no relevant mutations were identified were the strains in question designated as gWT).

## 2.3 Shape of MIC distributions

For each study, the shape of the corresponding MIC distribution was assessed based on five main criteria:

1. Inclusion of a control strain (i.e. typically a susceptible control strain, such as H37Rv for which the ATCC number was noted wherever possible since differences between variants have been observed[6]).
2. Whether the lower or upper end of the MIC distribution was truncated. For example, most studies did not test sufficiently low concentrations to define the lower end of the pWT MIC distribution for a given drug. Depending on the degree of truncation, this can make it difficult or, at times, impossible to establish the upper end of the pWT MIC distribution (i.e. the ECOFF).[7] In this context, it should be noted that defining the upper end of an individual distribution can be subjective, and multiple studies should ideally be combined in order to recommend a CC.
3. The consistency of the modes of the pWT MIC distribution (i.e. the most frequent MIC value in an individual MIC distribution). If the mode of an individual dataset differs by more than one two-fold dilution from the most common mode for an antibiotic on the same medium, this may indicate differences in MIC testing methodology.[7] This is particularly important given that, unlike for most other major bacterial pathogens, no reference method exists for MTBC. Consequently, differences in how stock solutions of drugs or bacterial inocula are prepared, as well as differences in other steps, may affect the comparability of data even on the same medium.
4. The overall number of pWT isolates tested.
5. Whether repeat MIC testing of individual isolates was done. Repeat testing can provide valuable information regarding the intra-laboratory reproducibility of MIC testing (particularly for the H37Rv reference strain). To capture this information, columns with the 'total [number of] MICs' vs. 'unique isolates' were included in the Excel datasheets. Entries that differed between these two columns were highlighted in red to designate isolates that were tested at least twice.

# 3 Additional comments regarding resistance mechanisms

## 3.1 SLIs

Resistance to KAN, AMK and CAP can arise through point mutations in the gene encoding the ribosomal 16S RNA subunit (*rrs* (*MTB000019*)).[8] An alteration in the ribosomal S23 RNA subunit (*rrl* (*MTB000020*)) has also been implicated in CAP mono-resistance *in vitro* (this mutation is not in the *rrl* region involved in LZD resistance and thus cross-resistance between the two drugs is unlikely (see Section 3.4)).[9] This CAP resistance mechanism has not been confirmed, to date, in clinical isolates. KAN mono-resistance is generally conferred by mutations in the aminoglycoside acetyltransferase Eis (Rv2416c) promoter, or by mutations in the 5' untranslated region of *whiB7* (*Rv3197A*), which encodes the positive regulator of *eis*.[10,11] *whiB7* mutations also confer low-level streptomycin cross-resistance in strains that have a functional copy of the Tap (Rv1258c) efflux pump.[12] The *whiB7* transcriptional start site reported by Reeves *et al.*, who first demonstrated the role of *whiB7* mutations in KAN resistance, is misannotated (i.e. the site is actually one nucleotide upstream of the location shown in Figure 1 of the respective publication[11]). For the purposes of this report, the Reeves *et al.* nomenclature was therefore updated to that of Burian *et al.*[13] Finally, CAP mono-resistance is conferred by loss-of-function mutations in the 2'-O-methyltransferase encoded by *tlyA* (*Rv1694*).[9]

## 3.2 CFZ and BDQ

*atpE*

In 2005, changes in the F0 subunit of ATP synthase via mutations in *atpE* (*Rv1305*) were documented to confer BDQ resistance in *in vitro* MTBC mutants.[14] The first clinical *atpE* mutants were reported from the Russian Federation in 2017, by Zimenkov *et al.*[15]

*mmpR*

Using two *in vitro* mutants selected with CFZ, Hartkoorn *et al.* found that *mmp*R (*Rv0678*) mutations conferred cross-resistance to both BDQ and CFZ.[16] Moreover, they demonstrated that expressing the *mmp*R S63R mutant gene (from one of their *in vitro* mutants) in an H37Rv background resulted in partial resistance to BDQ and CFZ.[16] MmpR, which functions as a homo-dimer, is the repressor of the MmpS5-MmpL5 efflux pump.[17] LOF mutations in *mmp*R therefore result in low-level resistance to both drugs.

The role of MmpS5-MmpL5 in conferring BDQ and CFZ cross-resistance was confirmed experimentally by Andries *et al.* using quantitative proteome analysis to show that this pump was upregulated in *mmp*R mutants.[18] Moreover, the group demonstrated that the over-expression of MmpS5-MmpL5 in H37Rv resulted in elevated BDQ MICs. Using REMA as the testing method, they reported that *mmp*R mutations usually resulted in 4- to 8-fold BDQ MIC increases, whereas *atpE* mutations increased BDQ MICs 32-fold. They also infected mice with *mmp*R mutants that had been isolated from mice treated with BDQ and CFZ. They found that subsequent BDQ treatment reduced the CFU counts, but that the mutants were clearly less susceptible to the drug compared to the wild type H37Rv controls. Notably, increasing the BDQ dose 8-fold did not overcome this resistance phenotype in any of the mutants. In addition, the

use of the efflux pump inhibitor verapamil did not lead to statistically significant improvements in the treatment of these mutants, compared to BDQ mono-therapy.

From a diagnostic point of view, *mmpR* mutations are challenging for three reasons. First, they appear to result in only modest BDQ and CFZ MIC increases (this is particularly true for mutations that do not completely abolish gene function). Second, given that the gene is non-essential, the spectrum of resistance mutations in this gene is large (i.e. distinguishing natural polymorphisms from significant resistance mutations is difficult). Third, even when obvious LOF mutations are identified by sequencing, they would not confer phenotypic resistance in isolates that harbour an inactive version of the MmpS5-MmpL5. Although this latter scenario has not been observed to date, a precedent for this possibility has been observed with *whiB7* mutations, which do not confer streptomycin resistance if the tap efflux pump is inactive (as is the case for most Beijing strains).[12]

*pepQ*

Zhang *et al.* first implicated the putative cytoplasmic peptidase encoded by *pepQ* (*Rv2535c*) in conferring CFZ resistance based on *in vitro* selection experiments.[19] Almeida *et al.* later observed that *pepQ* mutants that arose following the treatment of mice with BDQ (with or without CFZ) had MICs for BDQ and CFZ four times higher than the parental control.[20] The complementation of one of the mutants with wild type *pepQ* restored drug susceptibility both in *in vitro* and mice infection experiments. The fact that *pepQ* is also non-essential for survival of the organism raises similar challenges as for *mmpR* in the interpretation of molecular diagnostic test results.

*Rv1979c*

A mutation in *Rv1979c*, which encodes a possible permease, was noted in a single *in vitro* selected, CFZ-resistant strain (BDQ was not tested in this study).[19] A mutation in this gene has been recently correlated with CFZ resistance in one clinical isolate (the isolate remained susceptible to BDQ).[21] No detailed mechanistic work for this non-essential resistance gene has been published to date.[22] *Rv1979c* is deleted in the subgroup of BCG vaccine strains that share the RD2 deletion, but the effect of this change on CFZ or BDQ has not been investigated to date.[23,24]

Natural resistance

Isolates with *mmpR* or *Rv1979c* mutations with elevated MICs to BDQ and/or CFZ have been observed in patients without documented, prior exposure to either drug, raising the possibility that there might be an elevated rate of baseline resistance to both agents.[21,25-27] Confirmatory MIC testing of some of these isolates would be desirable to evaluate this possibility. Moreover, detailed phylogenetic analyses of these mutants, ideally using WGS, would be required to investigate how deeply rooted these mutations are in the MTBC phylogeny and how widespread natural resistance might be globally.

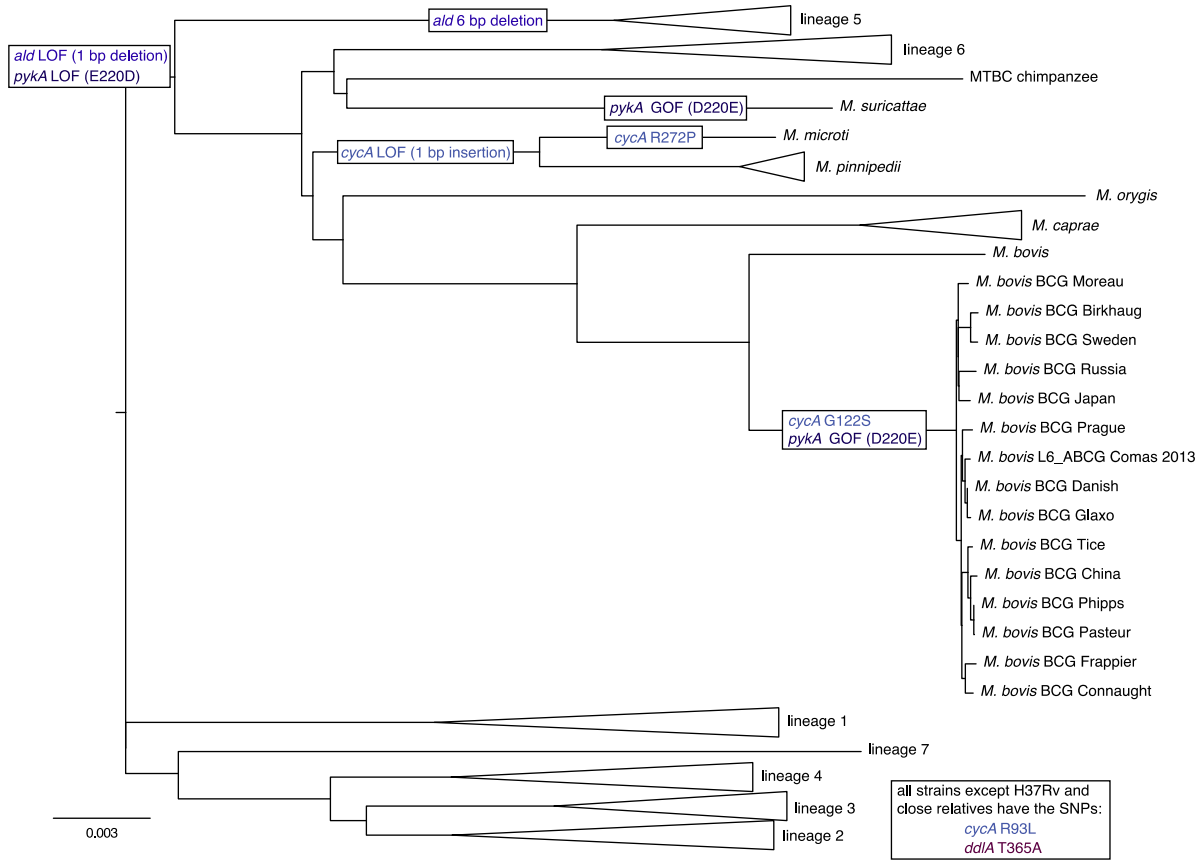
## 3.3 DCS

DCS is a cyclic analogue of D-alanine.[28] Consequently, DCS DST must be performed with a medium that does not contain D-alanine.[29,30] There is likely cross-resistance between DCS and TRD, as the latter compound combines two DCS molecules, but no MIC data confirming this hypothesis have been published to date.

It is unclear if alanine racemase (Alr (Rv3423c)) or D-alanine–D-alanine ligase (DdlA (Rv2981c)) is the primary target of DCS, but only Alr mutations have been shown to correlate with acquired resistance.[31-33] Moreover, mutations in *ald* (*Rv2780*), which encodes L-alanine dehydrogenase, have been implicated in both acquired and intrinsic DCS resistance.[32] The mechanism for this phenotype is believed to be the inability of *ald* mutants to convert L-alanine to pyruvate, which would increase the pool of L-alanine and therefore counteract competitive inhibition by DCS.[32]

The *Mycobacterium bovis* BCG vaccine strain is intrinsically resistant to DCS.[34] Chen *et al.* have demonstrated that the G122S mutation in *cycA* (*Rv1704c*) only partially explains this phenotype.[28] Desjardins *et al.* have proposed that an *ald* frameshift could contribute to the intrinsic resistance of BCG.[32] However, the complementation of BCG with the wild type *ald* gene did not result in a change in the DCS MIC in this study, using the 10% LJ proportion method. Nevertheless, the complemented strain had a significant growth disadvantage compared to the unmodified, parental BCG strain in the presence of DCS, which suggested that the frameshift likely plays a role in the intrinsic DCS resistance of BCG. Notably, the *ald* frameshift in BCG is shared by the entire RD9 branch of MTBC, raising the possibility that both *M. africanum* lineages as well as all animal strains might have elevated MICs compared to *M. tuberculosis* (Figure 1). However, more data are required to confirm this hypothesis, as *M. bovis* was the only RD9 strain tested by Desjardins *et al.*[32]

**Figure 1.** Maximum-likelihood phylogeny of MTBC




Maximum-likelihood phylogeny of MTBC with loss-of-function (LOF), gain-of-function (GOF), and other mutations in DCS resistance genes (noted in blue and purple).[32] The pyruvate kinase PykA (Rv1617), which shuttles pyruvate to Ald, is also shown in this figure, although the impact of LOF and GOF mutations in this gene have yet to be confirmed. Isolates of the RD9 branch (i.e. *M. africanum* and the animal species) likely have elevated MICs compared to *M. tuberculosis* due to an *ald* frameshift, although more data will be required to confirm this hypothesis (only *M. bovis* has been tested to date, which had an MIC of 25 mg/L using the 10% LJ proportion method compared to the tentative ECOFF of 20 mg/L. BCG, meanwhile had an even higher MIC of 40-60 mg/L due to the presence of the *cycA* G122S mutation).[32]

## 3.4 LZD

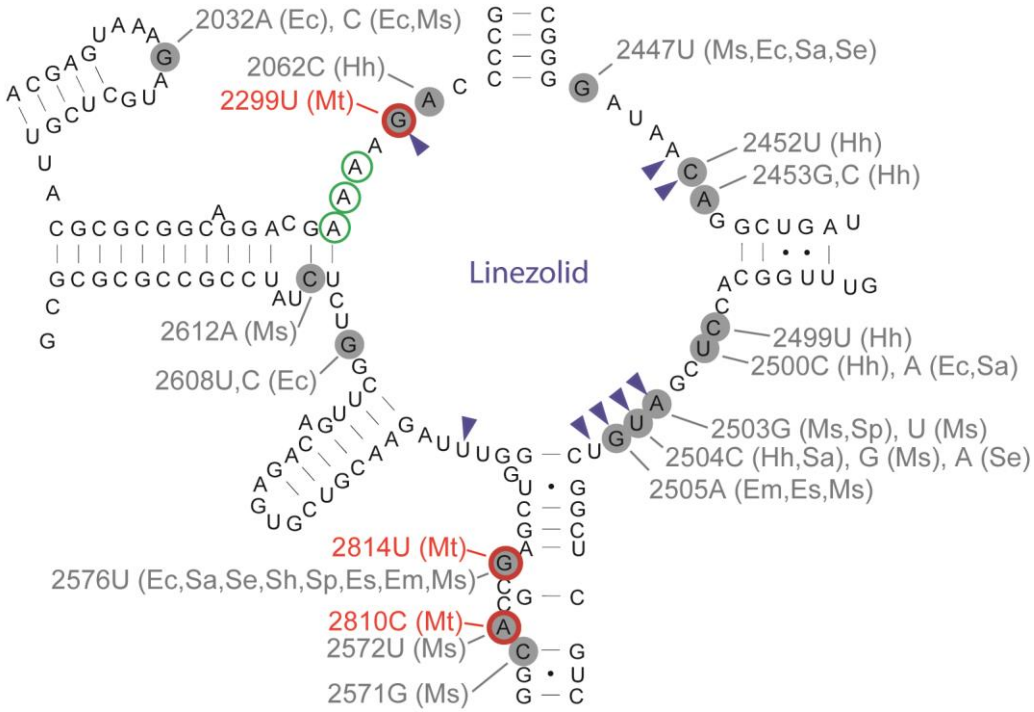
The genetic basis of LZD resistance has not been as well characterized, partly due to the fact that LZD resistance is rarely reported in MTBC. To date, only two genes have been implicated in LZD resistance based on *in vitro* and clinical association data from multiple laboratories: i) the 23S rRNA gene, *rrl* (*MTB000020*), and ii) the *rplC* (*Rv0701*) gene, which encodes the 50S ribosomal protein L3.[15,35-40] Of these resistance mechanisms, the C154R *rplC* mutation has been confirmed to result in elevated LZD MICs in recombinant *M. tuberculosis*.[41]

There is likely complete cross-resistance in MTBC between LZD and sutezolid (PNU-100480), which has undergone testing in phase IIa trials for the treatment of TB.[41,42] Cross-resistance also extends to AZD5847, although further clinical development of this drug for TB treatment has been discontinued.[42,43]

A single study by Johansen *et al.* has suggested that mutations in *rrl* also confer CAP resistance *in vitro*,[9] though this finding has not been confirmed in clinical isolates. It should also be noted that the *rrl* deletion observed by Johansen *et al.* was located in a different gene region than those mutations conferring LZD resistance (Figure 2).[9] Based upon these observations, cross-resistance between LZD and CAP is unlikely.

Clarithromycin targets the same *rrl* loop as LZD, which may explain the *in vitro* synergy observed between these agents.[44,45] However, only distinct mutations have been demonstrated to confer resistance to either drug (Figure 2), which means that cross-resistance between clarithromycin and LZD is unlikely. Even if cross-resistance between these compounds were to occur, the selective pressure on MTBC by clarithromycin would be low, as the drug is no longer recommended for the treatment of TB. Moreover, most MTBC isolates are intrinsically resistant to clarithromycin due to the action of the methyltransferase encoded by *ermMT* (this enzyme is functional and expressed at high levels in most MTBC, resulting in resistance to macrolides by modification of 23S rRNA, meaning that clarithromycin resistance-conferring *rrl* mutations are less likely to be selected for).[1,46]

**Figure 2.** Secondary structure of the peptidyl transferase loop of domain V of 23S rRNA.




Secondary structure of the peptidyl transferase loop of domain V of 23S rRNA (*M. tuberculosis* sequence).[47] The nucleotides that form the LZD binding pocket are indicated by blue triangles. Nucleotide changes that result in MIC increases for LZD in a variety of organisms are marked in grey (the *E. coli* numbering system is used in these cases). The corresponding organisms are indicated with two-letter abbreviations: Ec (*E. coli*), Sa (*S. aureus*), Se (*S. epidermidis*), Sh (*S. haemolyticus*), Sp (*S. pneumoniae*), Es (*E. faecalis*), Em (*E. faecium*), Ms (*M. smegmatis*), Mt (*M. tuberculosis*), and Hh (*H. halobium*). The three *M. tuberculosis* LZD resistance mutations included in this report are marked by red circles (the *M. tuberculosis* nucleotide positions for these mutations are included in red). Mutations that confer macrolide resistance in other organisms are circled in green.[44] The deletion implicated in CAP resistance (at position 1916) affects a different region of *rrl* and is not depicted here.[9]

## 3.5 DLM

DLM is a prodrug that requires activation though the $F_{420}$ coenzyme-dependent bioreduction pathway. Therefore, any LOF mutation in five enzymes of this pathway (i.e. *ddn* (*Rv3547*), *fgd1* (*Rv0407*), *fbiA* (*Rv3261*), *fbiB* (*Rv3262*), and *fbiC* (*Rv1173*)), which total almost 6.4 kbp (excluding promoter regions), results in an elevated DLM MIC.[48,49] Additionally, there is likely complete cross-resistance between DLM and pretomanid, a nitroimidazole currently in phase 3 trials.[48,50,51]

*Mycobacterium canettii* has been demonstrated to have elevated MICs to pretomanid relative to MTBC.[52] Whether this is a general feature of all *M. canettii* strains, and whether this characteristic also applies to DLM, has yet to be determined (the genetic basis for this phenotype is unclear and pretomanid MICs for only two *M. canettii* strains have been published to date). However, even if *M. canettii* is confirmed to have intrinsically elevated DLM MICs that preclude treatment with DLM, the impact of this phenomenon would be very limited as this specific species is rare globally.[53]

## 3.6 FQs

The vast majority of FQ resistance is caused by mutations in *gyrA* (*Rv0006*), which encodes the A subunit of the DNA gyrase.[54] Although appearing less frequently, mutations in the B subunit of the same enzyme (i.e. GyrB (Rv0005)) can also cause FQ resistance. Recently, Eilertson *et al.* implicated mutations in *eccC5* (*Rv1783*), involved in the ESX-5 secretion system, in OFX resistance.[55] However, this study used the resazurin microtiter assay system for MIC testing, and therefore did not meet the inclusion criteria for this review. Additional, independent studies are needed to confirm the findings of Eilertson *et al.* Therefore, for the purposes of this report, MIC data was stratified based on mutations reported in *gyrA* and *gyrB* only.

# 4 Supplementary tables for BDQ

**Table 2.** Relationship between baseline BDQ MICs and culture conversion rates.

| **Baseline BDQ MIC on 7H11 (mg/L)** | **BDQ treatment group 24-week culture conversion rate (%)** |
|---|---|
| ≤0.008 | 2/2 (100) |
| 0.015 | 15/18 (83.3) |
| 0.03 | 40/49 (81.6) |
| 0.06 | 80/105 (76.2) |
| 0.12 | 35/41 (85.4) |
| 0.25 | 1/2 (50.0) |
| 0.5 | 4/5 (80.0) |
| ≥1 | 0/1 (0) |

Presented data taken from the US Food and Drug Administration.[56]

**Table 3.** Baseline BDQ MICs compared to post-baseline BDQ MICs and treatment outcomes from BDQ clinical trial 209.

| TB type at BL | BL MIC | Post-BL MIC | MIC change | BL *mmpR* | Post-BL *mmpR* | Interim outcome | Final outcome |
|---|---|---|---|---|---|---|---|
| pre-XDR | 0.06 | 0.12 | <4 | gWT | mix | Failure to convert | Failure to convert |
| pre-XDR | 0.12 | 0.06 | <4 | not available | gWT | Response | Relapse |
| XDR | 0.06 | 0.06 | <4 | gWT | gWT | Failure to convert | Failure to convert |
| XDR | 0.03 | 0.03 | <4 | gWT | not available | Failure to convert | Failure to convert |
| XDR | 0.06 | 0.03 | <4 | gWT | gWT | Relapse | Response |
| pre-XDR | >0.5 | >0.5 | <4 | mix, Y92 frameshift | mix, W42R | Response | Response |
| XDR | 0.5 | 0.5 | <4 | S52F | S52F | Failure to convert | Response |
| MDR | 0.015 | 0.12 | ≥4 | C-11A | C-11A, N70I | Relapse | Relapse, death |
| pre-XDR | 0.06 | >0.5 | ≥4 | gWT | mix, T33A | Failure to convert | Failure to convert, Death |
| pre-XDR | 0.06 | 0.5 | ≥4 | gWT | R72 frameshift | Failure to convert | Failure to convert |
| pre-XDR | 0.06 | 0.5 | ≥4 | gWT | R156STOP | Failure to convert | Failure to convert |
| pre-XDR | 0.03 | 0.12 | ≥4 | not available | C46 frameshift | Relapse | Relapse |
| XDR | 0.015 | 0.06 | ≥4 | C-11A | C-11A mix, G66 frameshift | Failure to convert | Failure to convert |
| XDR | 0.06 | >0.5 | ≥4 | gWT | A36V | Failure to convert | Failure to convert |
| XDR | 0.06 | 0.5 | ≥4 | gWT | L143 frameshift | Response | Response |
| XDR | 0.06 | >0.5 | ≥4 | gWT | mix, IS6110 nt 172 frameshift | Failure to convert | Response |
| XDR | 0.06 | 0.5 | ≥4 | gWT | D47 frameshift | Relapse | Response |
| XDR | 0.06 | >0.5 | ≥4 | gWT | D47 frameshift | Failure to convert | Response |
| XDR | 0.06 | 0.25 | ≥4 | gWT | M139 frameshift | Response | Response |

All MICs are in mg/L and were tested on 7H11. Four-fold MIC increases were deemed potentially significant by Pym *et al.* in the analysis of trial C209.[57] Two isolates with elevated MICs at baseline are highlighted with bolded lines. Five post-baseline isolates that showed different MIRU-VNTR patterns to the baseline (BL) isolates from their respective patients. These pairs are consequently not included in this table. For the main report, the C-11A promoter mutations are classified as gWT and heteroresistant LOF mutants were shown as "other mutations".[27]

**Table 4.** Baseline BDQ MICs compared with post-baseline MICs for isolates that acquired *mmp*R or *atpE* mutations in Zimenkov *et al.*

| **Resistance profile** | **BL BDQ MIC** | **BL *mmpR*** | **Post-BL *mmpR*** | **Post-BL MIC (mg/L)** | **Post-BL *atpE*** |
|---|---|---|---|---|---|
| XDR | 0.03 | | 0.06 | Ins44a | |
| XDR | 0.03 | | 0.12 | M23L Ins419g | |
| poly | 0.03 | | 0.12 | (Del19g) (E49stop) (Del198g) (Ins468ga) | |
| MDR | 0.03 | | 0.12 | V85A | |
| XDR | 0.03 | | 0.12 | (F79S) (Ins137g) | |
| pre-XDR | 0.03 | | 0.12 | | D28N |
| XDR | 0.03 | | 0.25 | Ins139g | |
| XDR | 0.03 | | 0.25 | Del291c | |
| XDR | 0.06 | | 0.25 | (Ins144c) | |
| pre-XDR | 0.06 | | 0.25 | (Del435t) | |
| XDR | 0.06 | | 0.06 | (Del214c) (Del198g) | |
| XDR | 0.25 | L142R | 1.00 | L142R | A63V |

All baseline (BL) and post-baseline (post-BL) MICs are in mg/L and were tested on 7H11.[15] Unless otherwise stated, all sequences were gWT.

**Table 5.** Repeat MGIT BDQ MIC testing results for Ismail *et al.*

| Type | Initial BDQ MIC | Repeat BDQ MIC | *mmpR* mutation | Prior BDQ exposure |
|---|---|---|---|---|
| XDR | 4 | 4 | | |
| XDR | 4 | 1 | Glu49fs | Yes |
| Rif-R | 4 | 1 | Ser53Leu | |
| Rif-R | 2 | 2 | Gln51Pro | |
| XDR | 2 | 1 | | |
| Pre-XDR | 2 | 0.5 | | |
| Pre-XDR | 2 | 0.25 | | Yes |
| Pre-XDR | 2 | 0.25 | | Yes |
| Rif-R | 1 | 0.5 | | |
| MDR | 1 | 0.25 | | |
| XDR | 1 | 0.25 | | |
| Rif-R | 1 | ≤0.12 | | |
| MDR | 0.5 | 0.5 | | |
| Pre-XDR | 0.5 | 0.5 | | |
| MDR | 0.5 | 0.5 | | |
| MDR | 0.5 | 0.5 | | |
| Rif-S | 0.5 | 0.25 | | |
| MDR | 0.5 | 0.25 | | |
| MDR | 0.5 | 0.25 | | |
| Pre-XDR | 0.5 | 0.25 | | |
| XDR | 0.5 | 0.25 | | Yes |
| XDR | 0.5 | ≤0.12 | | |
| Rif-S | 0.5 | ≤0.12 | | |
| MDR | 0.5 | ≤0.12 | | |
| Pre-XDR | 0.25 | 0.5 | | |

All MICs are in mg/L. The repeat MICs are not included in the accompanying Excel datasheets. Unless otherwise stated, the isolates were not from patients with prior BDQ exposure and were all *mmpR* gWT.

# 5 Overview of literature review

## 5.1 Search terms

The following search terms were used to query PubMed to identify MIC data (date of last search 28.2.2017). The corresponding PRISMA diagrams can be found in Section 5.2 of this supplement.

SLIs (KAN, AMK and CAP):
"((aminoglycoside OR aminoglycosides OR (cyclic peptide) OR (cyclic peptides) OR kanamycin OR amikacin OR capreomycin) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)) OR ((tuberculosis OR TB) AND (rrs OR eis OR whiB7 OR tlyA OR rrl))"

CFZ:
"(clofazimine) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)"

BDQ:
"(bedaquiline OR sirturo OR TMC207 OR TMC-207 OR (TMC 207) OR R207910) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)"

DCS and TRD:
"(cycloserine OR terizidone) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance

levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)"

LZD:
"((linezolid) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)) OR ((tuberculosis OR TB) AND (rrl OR rplC))"

DLM:
"(delamanid OR OPC-67683 OR OPC67683 OR (OPC 67683) OR deltyba) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)"

FQs (OFX, LFX, GFX and MFX):
"((quinolone OR quinolones OR fluoroquinolone OR fluoroquinolones OR FQ OR FQs OR ofloxacin OR levofloxacin OR moxifloxacin OR gatifloxacin) AND (tuberculosis OR TB) AND (MIC OR MICs OR (minimum inhibitory concentration) OR (minimum inhibitory concentrations) OR (minimal inhibitory concentration) OR (minimal inhibitory concentrations) OR (critical concentration) OR (critical concentrations) OR concentration OR concentrations OR (resistance level) OR (resistance levels) OR level OR levels OR breakpoint OR breakpoints OR vitro OR vivo OR activity OR activities OR resistance OR resistances OR resistant OR susceptibility OR susceptibilities OR susceptible OR mutation OR mutations OR deletion OR deletions OR insertion OR insertions OR outcome OR outcomes)) OR ((tuberculosis OR TB) AND (gyrA OR gyrB))"

## 5.2 PRISMA diagrams for individual drug groups

A list of the PubMed IDs for each of the following diagrams can be found in the Excel 'PMIDs PRISMA diagrams v 1_4' file.

**Figure 3.** SLIs: Search results and exclusion criteria.

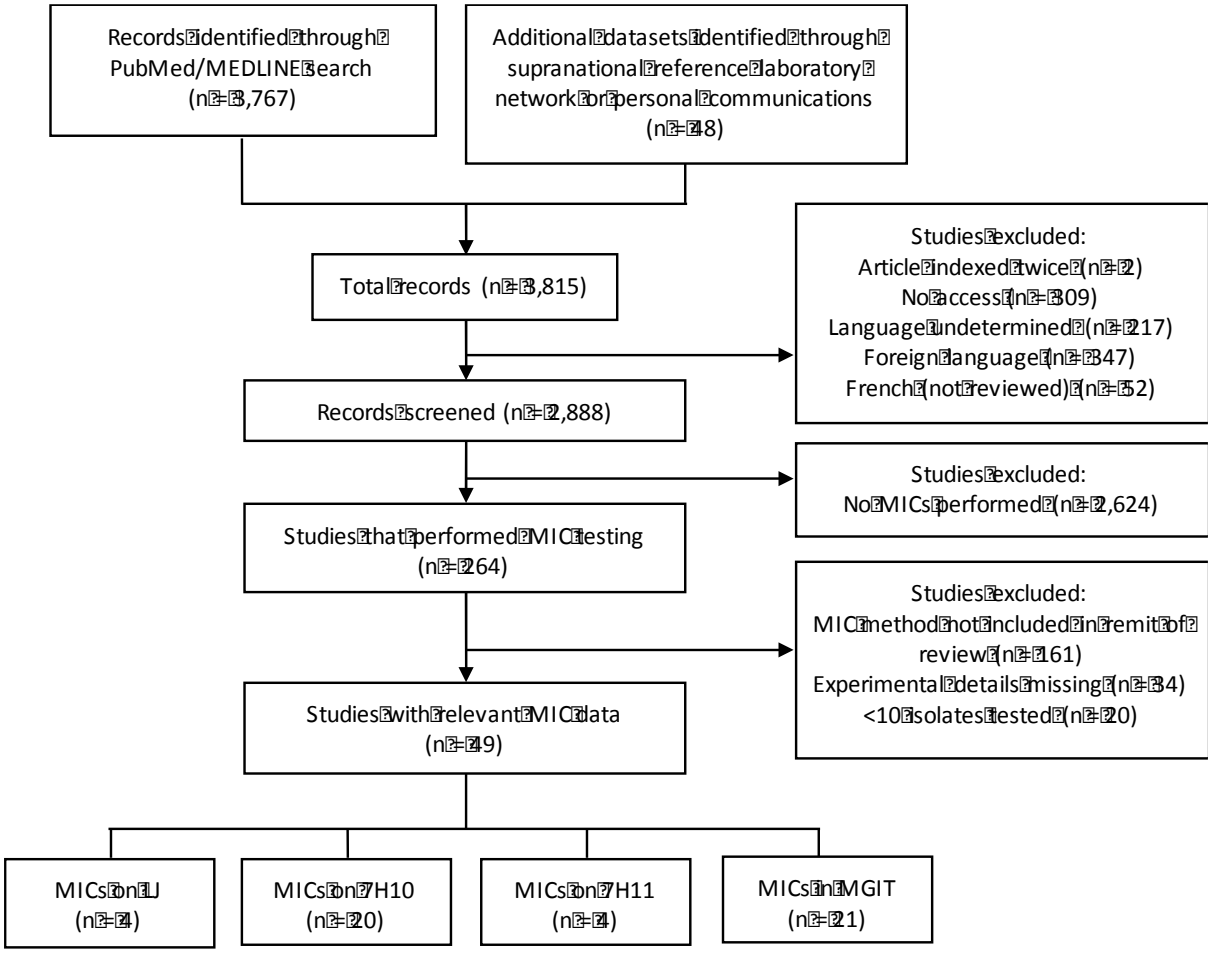


**Figure 4.** CFZ: Search results and exclusion criteria.

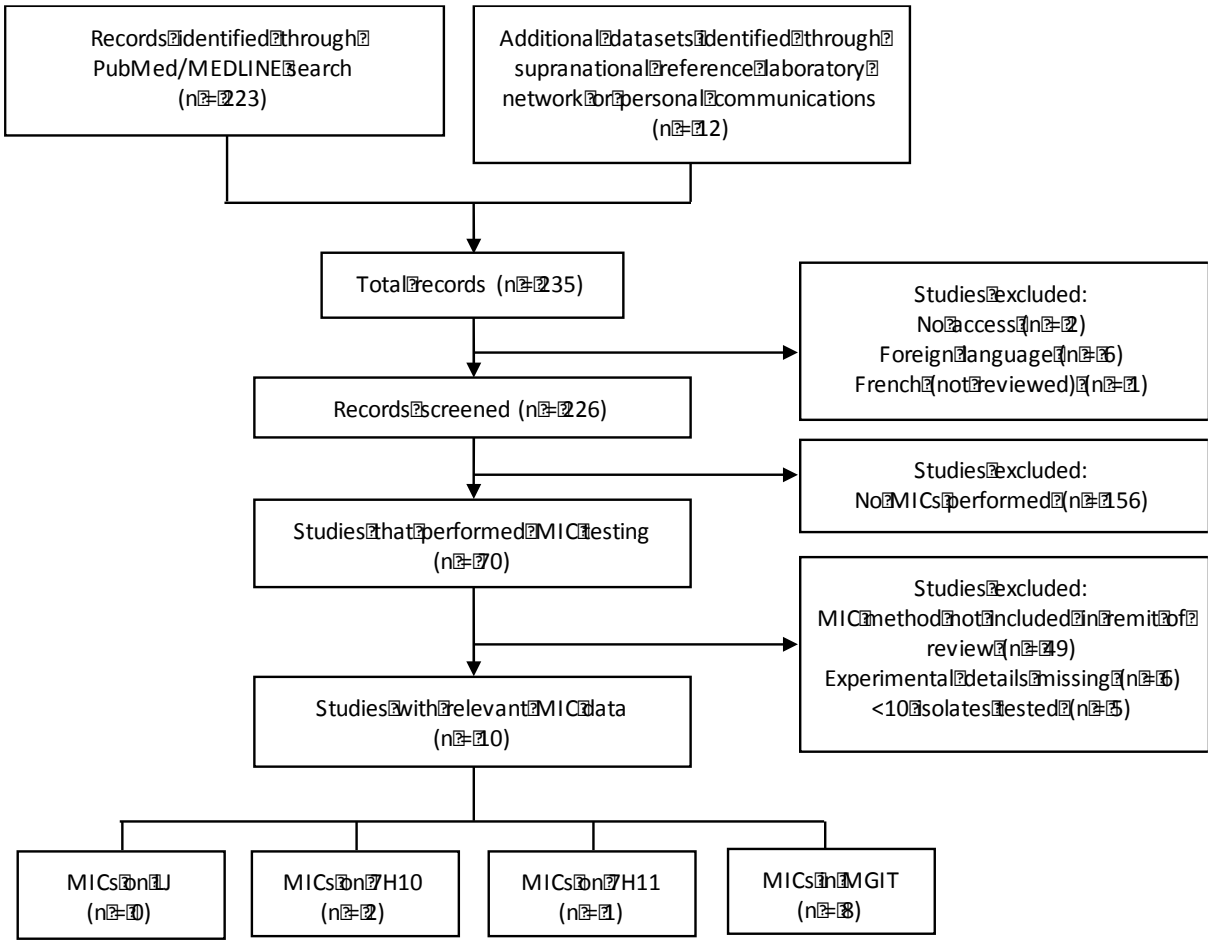


**Figure 5.** BDQ: Search results and exclusion criteria.

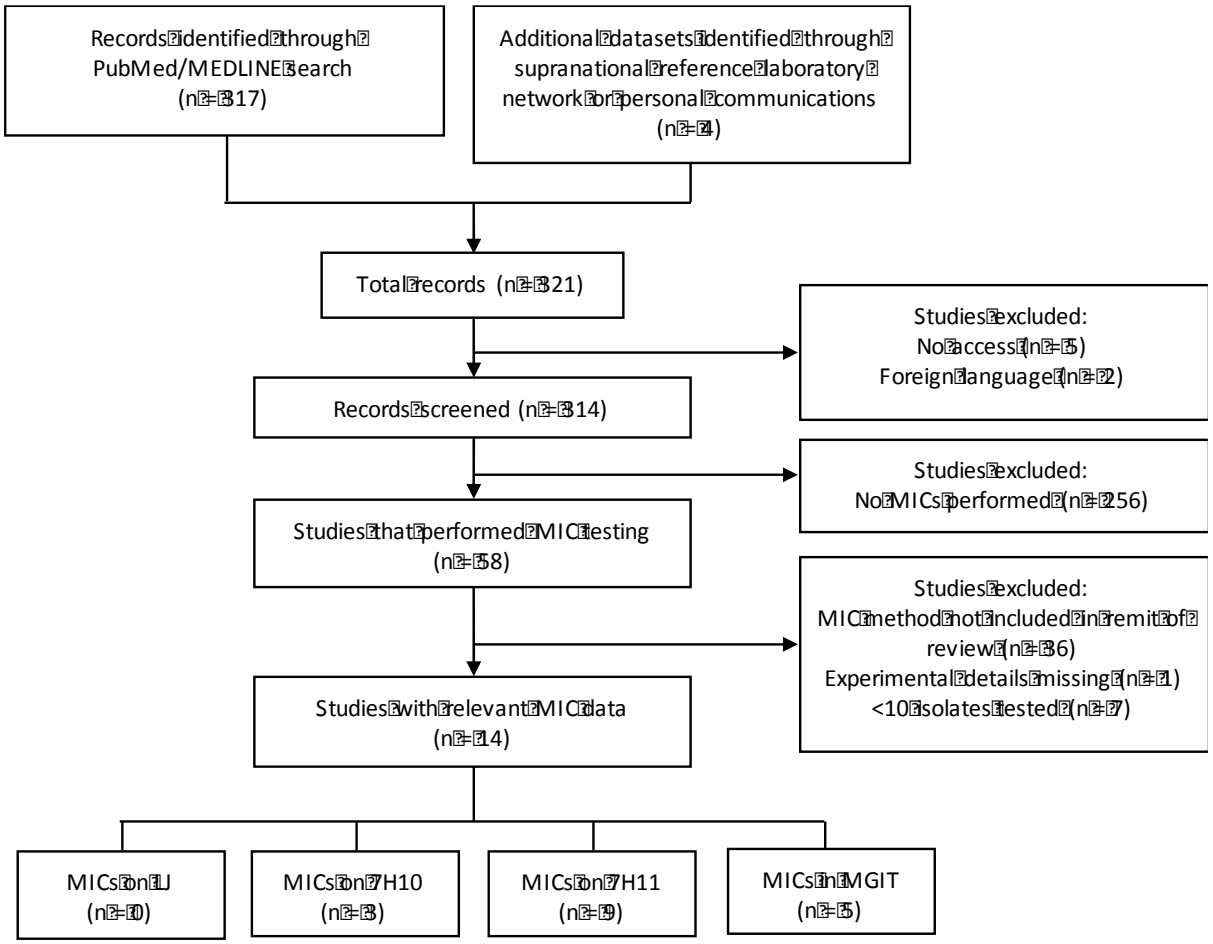


**Figure 6.** DCS and TRD: Search results and exclusion criteria.

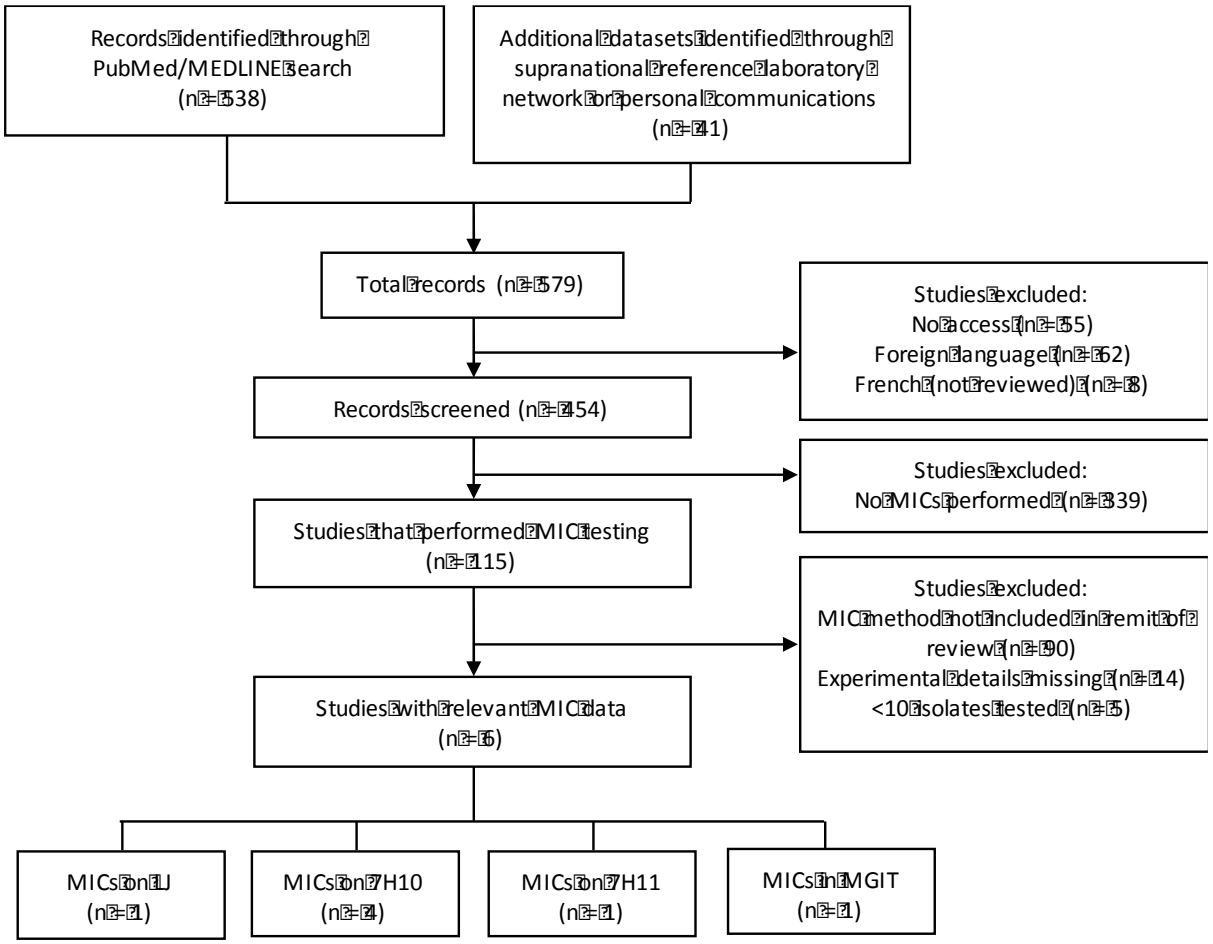


**Figure 7.** LZD: Search results and exclusion criteria.

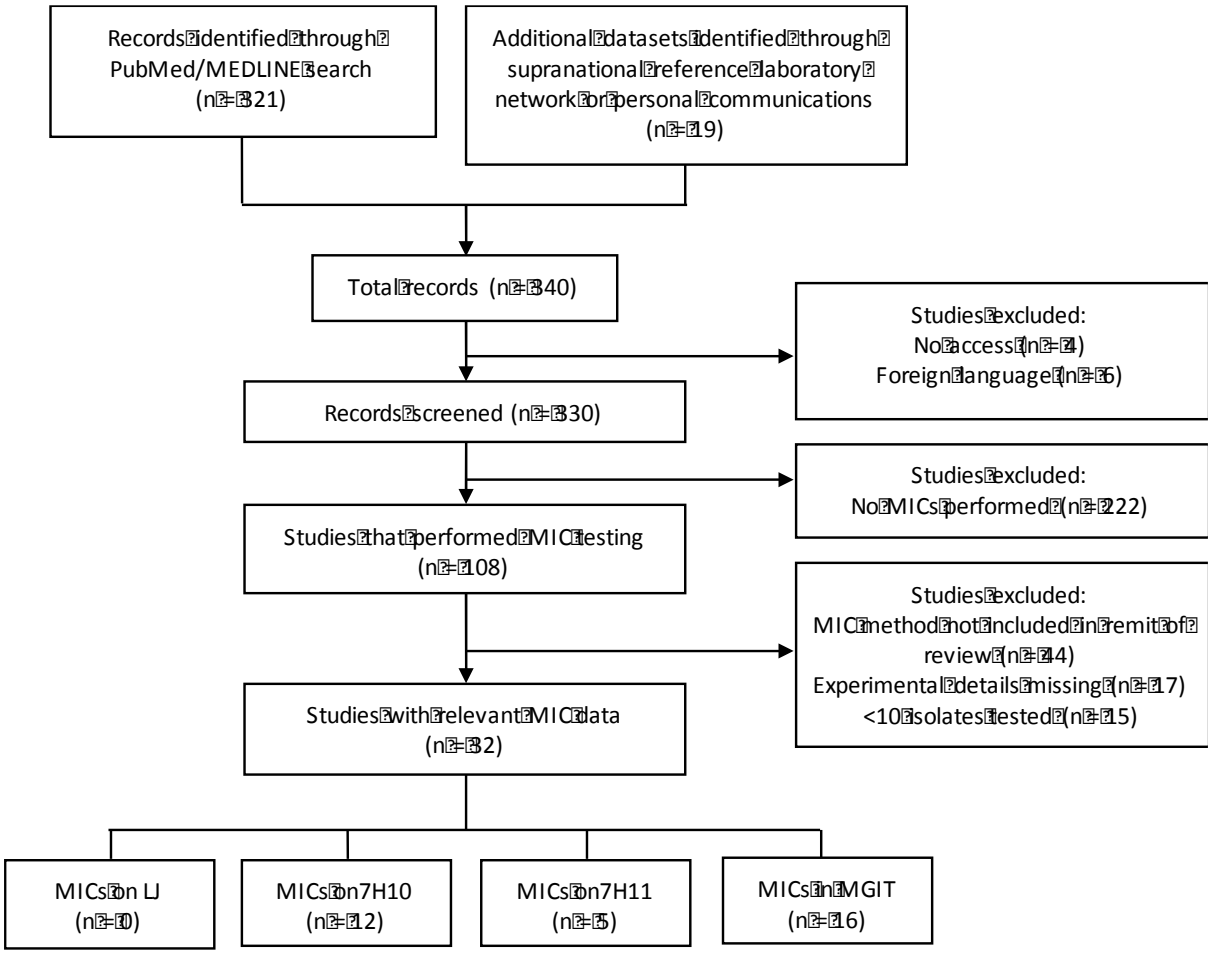


**Figure 8.** DLM: Search results and exclusion criteria.

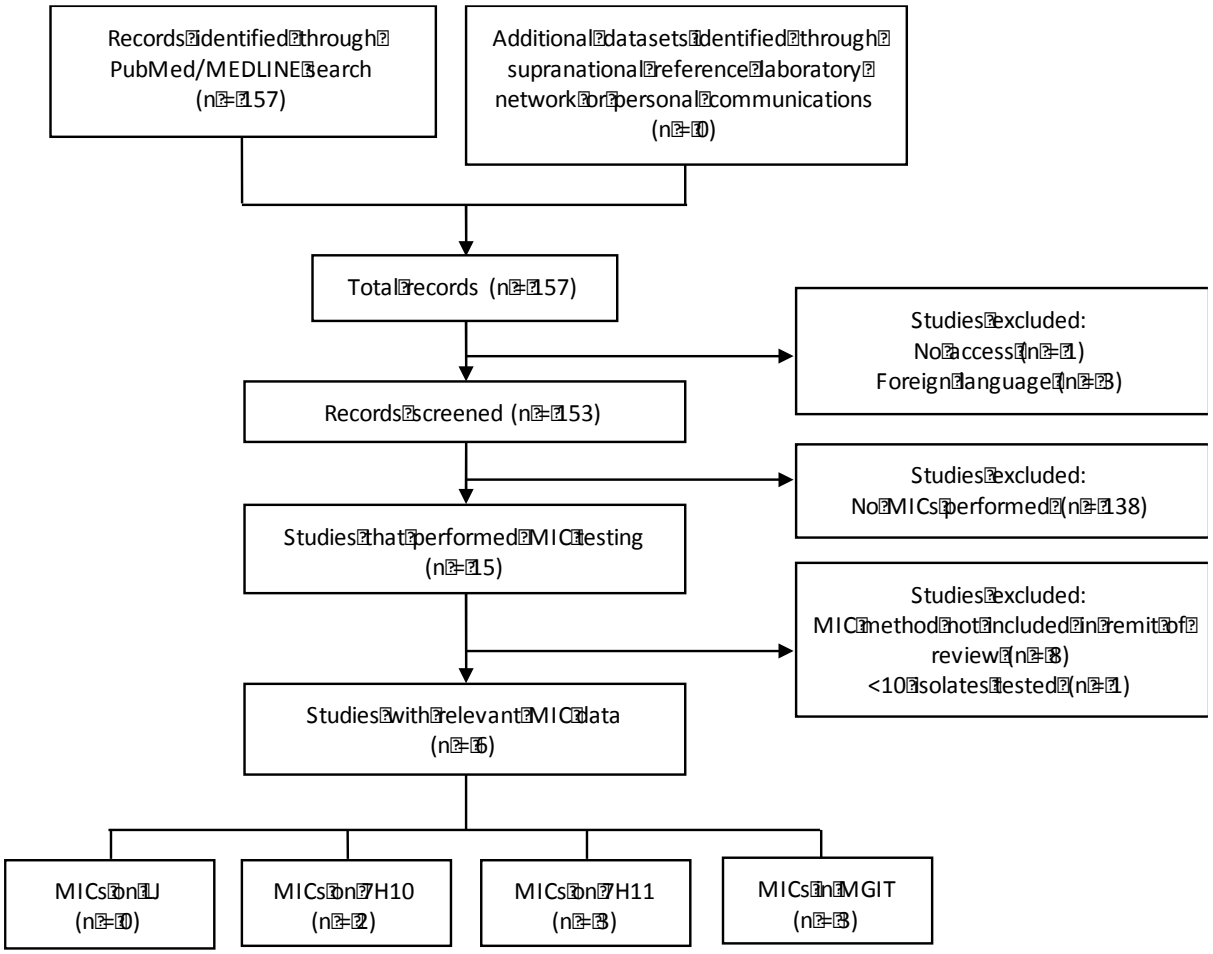
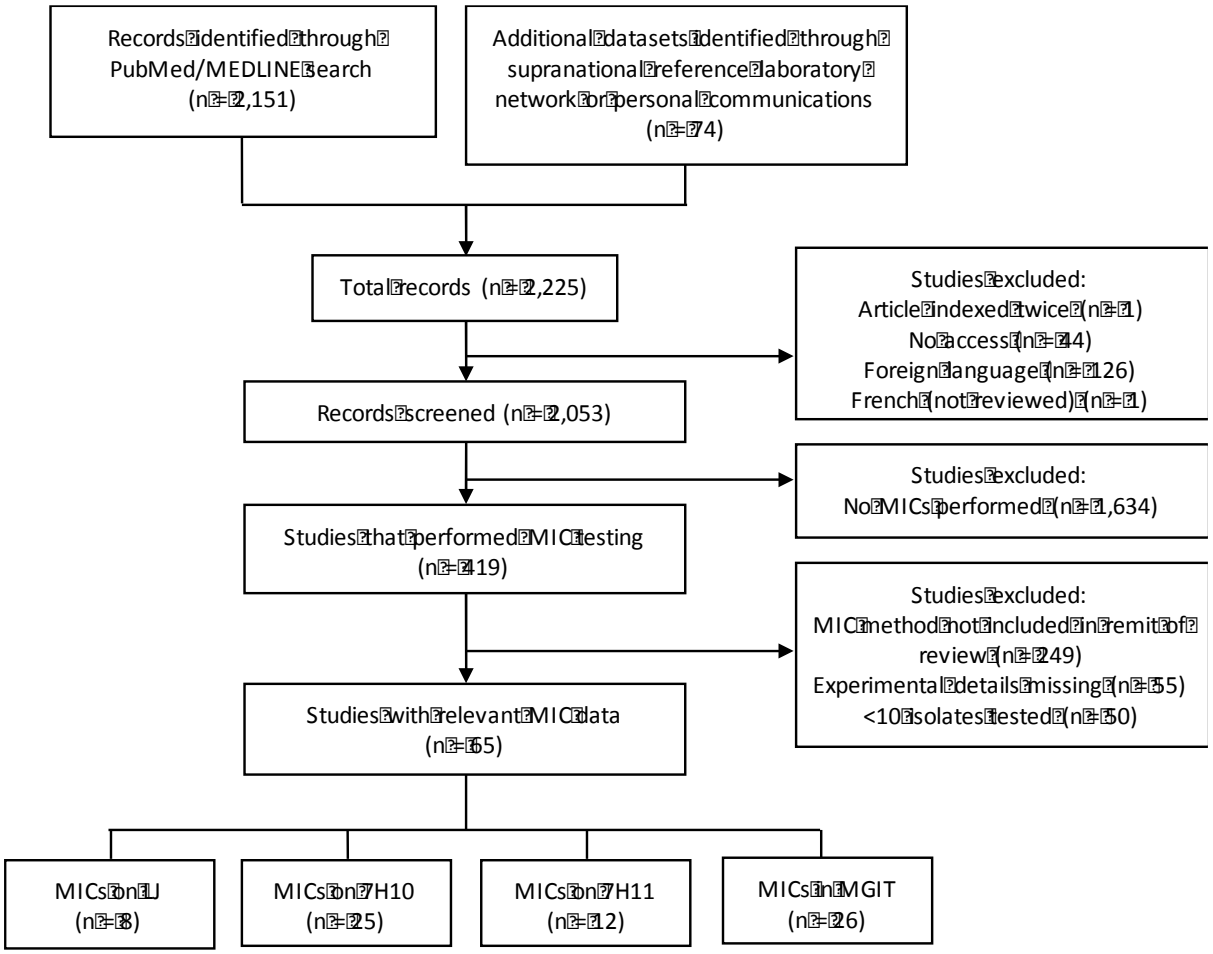


**Figure 9.** FQs: Search results and exclusion criteria.



# References


1. Köser, C.U., Feuerriegel, S., Summers, D.K., Archer, J.A. & Niemann, S. Importance of the genetic diversity within the *Mycobacterium tuberculosis* complex for the development of novel antibiotics and diagnostic tests of drug resistance. *Antimicrob Agents Chemother* **56**, 6080-7 (2012).
2. 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).
3. Kambli, P. *et al.* Correlating minimum inhibitory concentrations of ofloxacin and moxifloxacin with *gyrA* mutations using the Genotype MTBDR*sl* assay. *Tuberculosis (Edinb)* **95**, 137-41 (2015).
4. Zimenkov, D.V. *et al.* Detection of second-line drug resistance in *Mycobacterium tuberculosis* using oligonucleotide microarrays. *BMC Infect Dis* **13**, 240 (2013).
5. 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).
6. Ioerger, T. *et al.* Variation among genome sequences of H37Rv strains of *Mycobacterium tuberculosis* from multiple laboratories. *J Bacteriol* **192**, 3645-53 (2010).
7. European Committee for Antimicrobial Susceptibility Testing. EUCAST Subcommittee on MIC distributions and epidemiological cut-off values (ECOFFs). Discussion document Version 3, 9 March 2017.
8. Reeves, A.Z., Campbell, P.J., Willby, M.J. & Posey, J.E. Disparities in capreomycin resistance levels associated with the *rrs* A1401G mutation in clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **59**, 444-9 (2015).
9. Johansen, S.K., Maus, C.E., Plikaytis, B.B. & Douthwaite, S. Capreomycin binds across the ribosomal subunit interface using *tlyA*-encoded 2'-O-methylations in 16S and 23S rRNAs. *Mol Cell* **23**, 173-82 (2006).
10. Zaunbrecher, M.A., Sikes, R.D., Jr., Metchock, B., Shinnick, T.M. & Posey, J.E. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase *eis* confers kanamycin resistance in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* **106**, 20004-9 (2009).
11. Reeves, A.Z. *et al.* Aminoglycoside cross-resistance in *Mycobacterium tuberculosis* due to mutations in the 5' untranslated region of *whiB7*. *Antimicrob Agents Chemother* **57**, 1857-1865 (2013).

12. Köser, C.U., Bryant, J.M., Parkhill, J. & Peacock, S.J. Consequences of *whiB7* (*Rv3197A*) mutations in Beijing genotype isolates of the *Mycobacterium tuberculosis* complex. *Antimicrob Agents Chemother* **57**, 3461 (2013).

13. Burian, J. *et al.* The mycobacterial transcriptional regulator *whiB7* gene links redox homeostasis and intrinsic antibiotic resistance. *J Biol Chem* **287**, 299-310 (2012).

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

15. 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).

16. Hartkoorn, R.C., Upekar, S. & Cole, S.T. Cross-resistance between clofazimine and bedaquiline through up-regulation of MmpL5 in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **58**, 2979-81 (2014).

17. Radhakrishnan, A. *et al.* Crystal structure of the transcriptional regulator *Rv0678* of *Mycobacterium tuberculosis*. *J Biol Chem* **289**, 16526-40 (2014).

18. Andries, K. *et al.* Acquired resistance of *Mycobacterium tuberculosis* to bedaquiline. *PLoS One* **9**, e102135 (2014).

19. Zhang, S. *et al.* Identification of novel mutations associated with clofazimine resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* **70**, 2507-10 (2015).

20. Almeida, D. *et al.* Mutations in *pepQ* confer low-level resistance to bedaquiline and clofazimine in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **60**, 4590-9 (2016).

21. Xu, J. *et al.* Primary clofazimine and bedaquiline resistance among isolates from patients with multidrug-resistant tuberculosis. *Antimicrob Agents Chemother* **61**, e00239-17 (2017).

22. Lamichhane, G. *et al.* A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: application to *Mycobacterium tuberculosis*. *Proc Natl Acad Sci U S A* **100**, 7213-8 (2003).

23. Abdallah, A.M. *et al.* Genomic expression catalogue of a global collection of BCG vaccine strains show evidence for highly diverged metabolic and cell-wall adaptations. *Sci Rep* **5**, 15443 (2015).

24. Yew, W.W., Liang, D., Chan, D.P., Shi, W. & Zhang, Y. Molecular mechanisms of clofazimine resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* **72**, 2943-2944 (2017).

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

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

27. 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).

28. Chen, J.M., Uplekar, S., Gordon, S.V. & Cole, S.T. A point mutation in *cycA* partially contributes to the D-cycloserine resistance trait of *Mycobacterium bovis* BCG vaccine strains. *PLoS One* **7**, e43467 (2012).

29. Meissner, G. On cycloserine. *Beitr Klin Tuberk Spezif Tuberkuloseforsch* **127**, 333-4 (1963).

30. Awasthy, D., Bharath, S., Subbulakshmi, V. & Sharma, U. Alanine racemase mutants of *Mycobacterium tuberculosis* require D-alanine for growth and are defective for survival in macrophages and mice. *Microbiology* **158**, 319-27 (2012).

31. Merker, M. *et al.* Whole genome sequencing reveals complex evolution patterns of multidrug-resistant *Mycobacterium tuberculosis* Beijing strains in patients. *PLoS One* **8**, e82551 (2013).

32. Desjardins, C.A. *et al.* Genomic and functional analyses of *Mycobacterium tuberculosis* strains implicate *ald* in D-cycloserine resistance. *Nat Genet* **48**, 544-51 (2016).

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

34. Canetti, G. Bacterial resistance and its relation to chemotherapy. *Z Tuberk Erkr Thoraxorg* **128**, 231-8 (1968).

35. Hillemann, D., Rüsch-Gerdes, S. & Richter, E. *In vitro*-selected linezolid-resistant *Mycobacterium tuberculosis* mutants. *Antimicrob Agents Chemother* **52**, 800-1 (2008).

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

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

38. Zhang, Z., Pang, Y., Wang, Y., Liu, C. & Zhao, Y. Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with linezolid resistance in multidrug-resistant and extensively drug-resistant tuberculosis in China. *Int J Antimicrob Agents* **43**, 231-5 (2014).

39. Perdigão, J. *et al.* Beyond extensively drug-resistant tuberculosis in Lisbon, Portugal: a case of linezolid resistance acquisition presenting as an iliopsoas abscess. *Int J Antimicrob Agents* (2016).

40. Zhang, S. *et al. Mycobacterium tuberculosis* mutations associated with reduced susceptibility to linezolid. *Antimicrob Agents Chemother* **60**, 2542-4 (2016).

41. Makafe, G.G. *et al.* Role of the Cys154Arg substitution in ribosomal protein L3 in oxazolidinone resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* **60**, 3202-6 (2016).

42. Lessem, E. The tuberculosis treatment pipeline: Activity, but no answers. (http://pipelinereport.org/2016/tb-treatment) accessed 15.12.2106. (2016).

43. Balasubramanian, V. *et al.* Bactericidal activity and mechanism of action of AZD5847, a novel oxazolidinone for treatment of tuberculosis. *Antimicrob Agents Chemother* **58**, 495-502 (2014).

44. Pfister, P. *et al.* 23S rRNA base pair 2057-2611 determines ketolide susceptibility and fitness cost of the macrolide resistance mutation 2058A-->G. *Proc Natl Acad Sci U S A* **102**, 5180-5 (2005).

45. Zou, L., Liu, M., Wang, Y., Lu, J. & Pang, Y. Determination of *in vitro* synergy between linezolid and other antimicrobial agents against *Mycobacterium tuberculosis* isolates. *Tuberculosis (Edinb)* **95**, 839-42 (2015).

46. Warit, S. *et al.* Genetic characterisation of a *whiB7* mutant of a *Mycobacterium tuberculosis* clinical strain. *J Glob Antimicrob Resist* **3**, 262-266 (2015).

47. Long, K.S. & Vester, B. Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrob Agents Chemother* **56**, 603-12 (2012).

48. 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).

49. Köser, C.U. *et al.* Drug-resistance mechanisms and tuberculosis drugs. *Lancet* **385**, 305-307 (2015).

50. Hurdle, J.G. *et al.* A microbiological assessment of novel nitrofuranylamides as anti-tuberculosis agents. *J Antimicrob Chemother* **62**, 1037-45 (2008).

51. Gurumurthy, M. *et al.* Substrate specificity of the deazaflavin-dependent nitroreductase from *Mycobacterium tuberculosis* responsible for the bioreductive activation of bicyclic nitroimidazoles. *FEBS J* **279**, 113-25 (2012).

52. Feuerriegel, S. *et al.* Impact of *fgd1* and *ddn* diversity in *Mycobacterium tuberculosis* complex on *in vitro* susceptibility to PA-824. *Antimicrob Agents Chemother* **55**, 5718-22 (2011).

53. Aboubaker Osman, D., Bouzid, F., Canaan, S. & Drancourt, M. Smooth tubercle bacilli: neglected opportunistic tropical pathogens. *Front Public Health* **3**, 283 (2016).

54. 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).

55. Eilertson, B. *et al.* A novel resistance mutation in eccC5 of the ESX-5 secretion system confers ofloxacin resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother* **71**, 2419-27 (2016).

56. Food and Drug Administration. Sirturo. Highlights of prescribing information. Reference ID 3237647. https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/204384s000lbl.pdf (accessed 22.4.2017).

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