Issues in *Mycobacterium tuberculosis* Complex Drug Susceptibility Testing:

Fluoroquinolones

Report Highlights

- Rapid and reliable detection of fluoroquinolone (FQ) resistance has become more important as short-course and all-oral treatment regimens, dependent on moxifloxacin (MFX) as a first-line therapy, have been described.
- Most FQ resistant *Mycobacterium tuberculosis* complex (MTBC) isolates contain mutations in the Quinolone Resistance Determining Region (QRDR) of the *gyrA* gene or, to a lesser extent, the QRDR of the *gyrB* gene.
- It is important to detect heteroresistance, the presence of both FQ susceptible and FQ resistant sub-populations within a single specimen. Sequencing-based methods may more readily detect heteroresistance than phenotypic methods.
- While cross-resistance between FQs can sometimes be predicted, it is incomplete. Some mutations confer varied levels of resistance to different FQs or no resistance when performing minimum inhibitory concentration (MIC) testing.
- Testing at least two concentrations of MFX may help differentiate low-level FQ resistance from high-level resistance. Strains possessing low-level FQ resistance may be treatable with MFX or high dose levofloxacin.

Contents

Background

Moxifloxacin (MFX), levofloxacin (LVX), ofloxacin (OFX), ciprofloxacin and gatifloxacin are broad-spectrum fluorinated carboxyquinolones—also known as fluoroquinolones (FQs)—drugs which, when used for the treatment of tuberculosis (TB), have traditionally been second-line medications for multidrug resistant (MDR) TB.[1] More recently, FQs have become essential components of new US Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) recommended shorter course regimens for treatment of drug susceptible TB and all-oral regimens for treatment of MDR or drug intolerant TB.[2–6] Due to its inclusion in the four month rifapentine-moxifloxacin regimen, MFX can now be considered a first-line therapy for tuberculosis and included as part of first-line drug susceptibility testing (DST) panels.

Moxifloxacin (MFX)

In 2022, CDC provided guidance for use of a four-month rifapentine-MFX treatment regimen for drug susceptible TB. With its inclusion in this short-course regimen, MFX may now be considered a first-line therapy for drug susceptible TB and included as part of first-line DST panels.

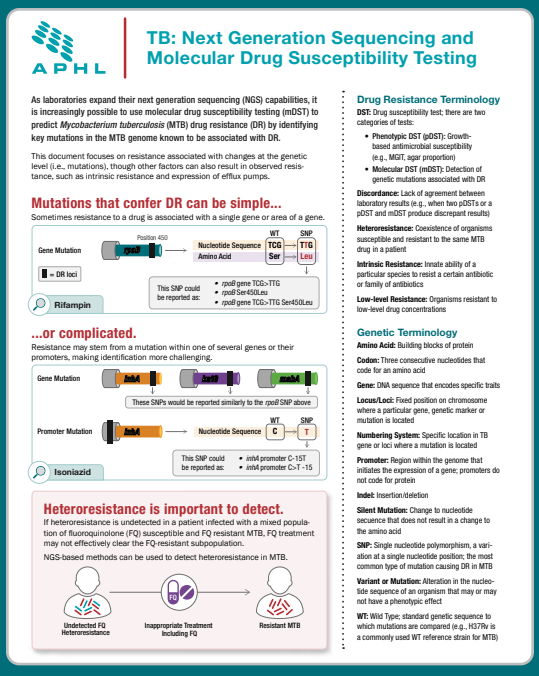
Both rapid molecular susceptibility testing and growth-based susceptibility testing with a panel that includes MFX are recommended as part of the four-month regimen.

The mechanism of action of FQs is to bind DNA gyrase (class II topoisomerases; *gyrA* and *gyrB*) in a concentration-dependent manner, leading to inhibition of DNA synthesis. This halts cellular reproduction, causing a bactericidal effect. In the United States, MFX and LVX are the FQ drugs used most often. While OFX has previously been used in treatment regimens for patients with drug resistance, it is no longer recommended nor frequently used in clinical practice.[7] Gatifloxacin is included in the WHO recommendations; however, use of this drug was discontinued in the US in 2006 due to a severe side effect[8] and is not discussed further in this paper. Ciprofloxacin is no longer recommended by WHO for the treatment of MDR-TB due to the lack of evidence of its effectiveness.[9]

Laboratories perform FQ growth-based DST using a variety of methods including agar proportion (AP), BACTEC™ MGIT™ system, VersaTREK® system (scheduled for discontinuation in 2029) or a Sensititre broth microdilution test. Each of these methods has advantages and disadvantages and each must be validated for clinical use. In terms of *in vitro* drug susceptibility, MFX is more potent than LVX *in vitro,*[10] however, outcomes using treatment regimens containing either drug have generally been similar.[11]

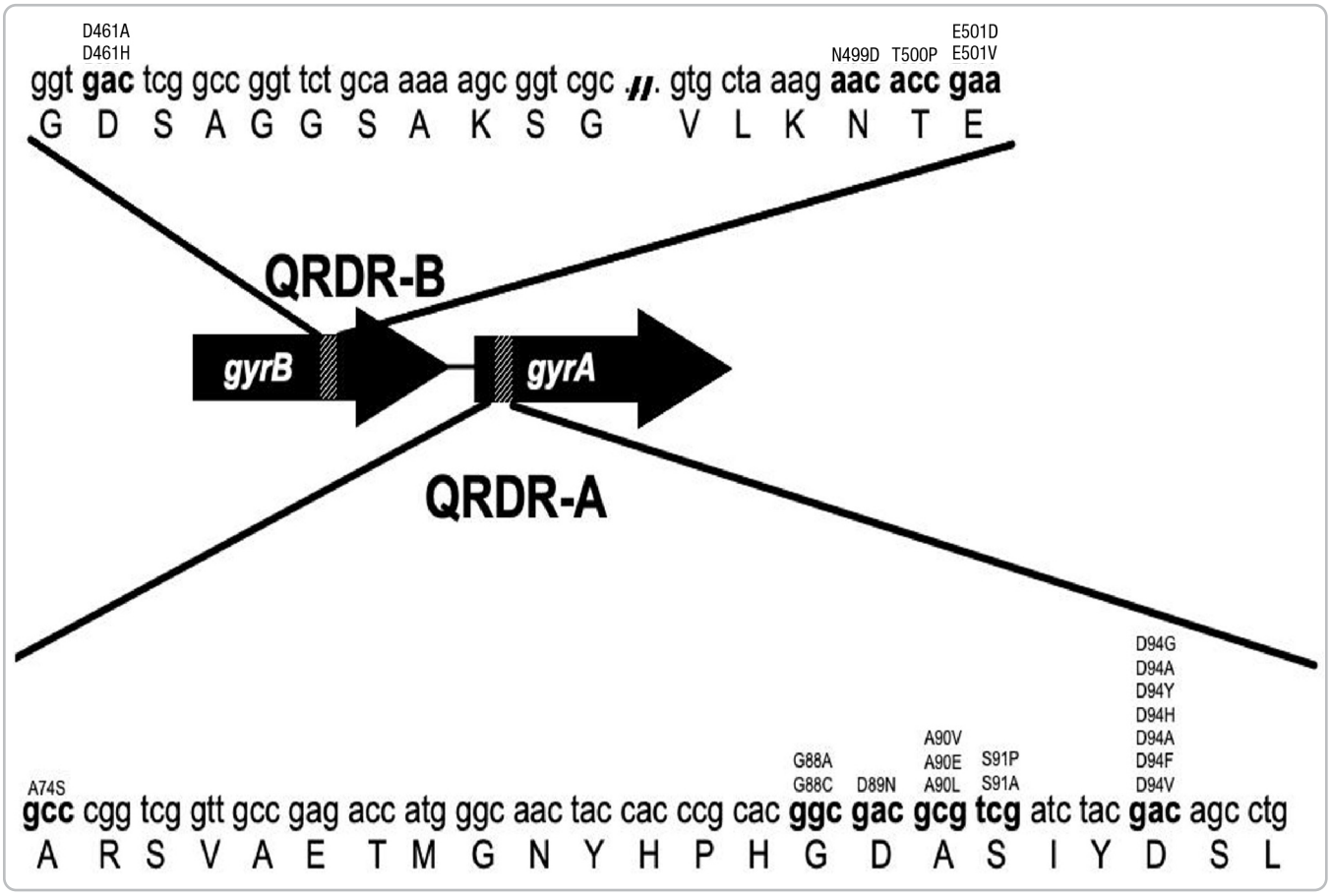
In addition to growth-based DST, molecular detection of mutations can help predict drug resistance, and is recommended when the four-month rifapentine-MFX regimen is used.[4] Resistance to FQ is frequently linked to mutations within the quinolone-resistance-determining region (QRDR) of DNA gyrase subunits *gyrA* and, to a lesser extent, *gyrB.*[12–14] The most common mutations associated with FQ resistance are located in codon 94 encoded by the *gyrA* gene; other implicated mutations at codons 88, 89, 90 and 91 are also well-documented.[13,15,16] Codons containing mutations encoded by the *gyrB* gene of the QRDR that have been attributed to FQ resistance include 447, 461, 499, 501 and 504[14–16] (**Figure 1, page 3**).

Learn more about TB drug-related mutations and molecular testing with APHL's fact sheet, "TB: Next Generation Sequencing and Molecular Drug Susceptibility Testing."



The issue of cross-resistance among the FQs is relevant to laboratorians and clinicians as it may impact DST results and contribute to the level of success of treatment regimens. In *Mycobacterium tuberculosis* complex (MTBC) strains, different mutations may confer varying levels of resistance to different FQs or no resistance when performing minimum inhibitory concentration (MIC) testing.[17,18] In addition, growth-based testing of multiple FQs may not be warranted due to known cross-resistance within the FQs or because DST is performed with the specific FQ that will be used in the treatment regimen.[19] CLSI recommends consultation with the treating physician to select at least one FQ for DST,[20] whereas WHO recommends evaluating each FQ used in a TB program treatment regimen and advises against using a single representative drug such as OFX.[19]

Figure 1. Diagram of the important mutations in the *gyrA* and *gyrB* genes conferring resistance to FQs



Practical Laboratory Issues

Growth-based Phenotypic FQ DST and Test Methods

AP methods are performed using either Middlebrook 7H10 or 7H11 agar and are considered the reference methods for FQ DST.[20] Determination of resistance is based on the number of colony-forming units (CFU) growing on the medium containing FQ at the critical concentrations compared with the number of CFU growing on the drug-free medium, with growth of > 1% in drug containing media indicating resistance. The main advantage of AP is the ability to detect heteroresistance, the presence of both drug-resistant and drug-susceptible subpopulations in a culture or clinical sample. The disadvantage to AP is the three-week incubation period before a final report is issued.

The BACTEC™ MGIT™ System (Becton Dickinson) and the VersaTREK™ Automated Microbial Detection System (ThermoFisher Scientific™) are commercial automated broth systems that require shorter incubation time than AP for susceptibility testing. van Ingen, et. al demonstrated high concordance between the AP method and MGIT for MFX with a breakpoint of 0.5 µg/mL.[21] Neither system is cleared by the US Food and Drug Administration (FDA) for testing FQs, but some laboratories have completed in-house validation studies to perform testing for MFX and OFX at critical concentrations of 0.25 and 2.0 µg/mL, respectively, as a laboratory developed test (LDT). Additionally, other researchers have demonstrated comparable findings for MFX[22] and OFX.[23,24] In 2018 the WHO recommended lowering the critical concentration and critical breakpoint for LVX to 1.0 µg/mL[7] in order to capture most low-level resistant mutations; current CLSI recommendations for LVX critical concentration remain at 1.5 µg/mL.[20] The application of these recommendations varies by institution and is discussed in further detail in the **"Growth-based DST" section (page 7)**.

A microtiter plate broth dilution system, Sensititre™ *Mycobacterium tuberculosis* MYCOTB Plate (ThermoFisher Scientific), henceforth referred to as Sensititre, has been developed for first and second-line anti-TB drugs. Considered a research use only (RUO) assay in the US, this assay can be validated as a LDT in accordance with FDA and CLIA regulations. The Sensititre plate includes MFX (concentration range from 0.06 to 8 µg/mL) and OFX (concentration range from 0.25 to 32 µg/mL). LVX is available on a customized Sensititre™ plate and testing using the concentration range from 0.06 - 8 µg/mL is suggested by the WHO.[25] The manufacturer's protocol requires inoculum preparation from solid media, followed by assessing the Sensititre plate for growth at 10 days post-inoculation.[26] Plates are read when sufficient growth is noted in the control wells. If growth is insufficient at day 10, plates can be reincubated for up to 11 additional days if needed.[26] Although there are no established interpretive breakpoints for resistance with this assay, MIC data can provide information regarding low level resistance, giving physicians an opportunity to adjust dosage of FQs in an effort to enhance therapeutic efficacy.

Molecular-based Genotypic FQ DST and Test Methods

Molecular-based genotypic assays provide rapid DST results for FQs compared to growth-based phenotypic methods. In the US, LDTs are the only available molecular testing methods for detection of mutations in the *gyrA* and/or *gyrB* genes associated with resistance to FQs. While laboratories may develop their own assays, some LDTs are commercially available as RUOs including the probe-based GenoType MTBDR*sl* assay (Hain LifeScience) and DNA sequencing assays, such as the AmpliSeq for Illumina TB Research Panel (Illumina), AmPORE TB® (Oxford Nanopore Diagnostics), QIAseq xHYB *Mycobacterium tuberculosis* Panel (Qiagen)[27] and the Deeplex® Myc-TB assay (Illumina/ Genoscreen). A benefit to using these molecular assays is that they can be performed rapidly and directly on a specimen or isolates. The incorporation of molecular-based assays into testing algorithms and for patient management has been described.[28–30]

DNA sequencing[18,31,32] can provide additional detail that probe-based assays cannot because the exact DNA sequence is determined and some sequencing methods may additionally allow for detection of heteroresistance. There are two general types of sequencing-based assays: targeted sequencing such as Sanger sequencing and targeted next generation sequencing (tNGS) and whole genome sequencing (WGS). Both WGS[33–36] and tNGS[37–39] approaches provide comprehensive analysis of the MTBC genome to assess molecular-based DST more accurately. However, when sequencing-based methods are used for DST, data analyses and interpretations must differentiate between mutations that are known to confer FQ resistance, those that are NOT known to predict resistance to FQ, and those that have an unknown effect on FQ resistance.[15,33] The WHO *Catalogue of Mutations in* Mycobacterium tuberculosis *Complex and their Association with Drug Resistance* is the first document to provide a common, standardized reference for the interpretation of resistance to first and second-line drugs.[15,16]

WHO's *Catalogue of Mutations in* Mycobacterium tuberculosis *Complex and Their Association with Drug Resistance*—first published in 2021, with a second edition in 2023—is the first document to provide a common, standardized reference for the interpretation of resistance to first- and second-line drugs. It includes 17,000 mutations, their frequency and association with resistance. The mutations are classified into two tiers:

- **Tier 1:** Genes considered most likely to contain the resistance mutations.
- **Tier 2:** Genes that are reasonably likely to contain resistance mutations, with their additional literature-defined promoter sequences.

The Catalogue is an important resource with detailed information on numerous mutations associated with drug resistance and may include mutations not specifically addressed in this white paper. We recommend readers to review both documents if they are interested in molecular detection of drug resistance.

Targeted NGS enables the detection of a significant portion of relevant mutations and could serve a similar role as WGS, with the added benefit that tNGS may be readily applied directly to specimens and provides results more quickly than WGS, often at a lower cost.[37–39] Studies describing the utility of tNGS performed directly from a specimen have been published,[37,39,40] including those using the commercially-available RUO products mentioned previously. The AmpliSeq for Illumina TB Research Panel (Illumina) provides full-length gene analysis using ion semiconductor NGS,[40,41] whereas the Deeplex® Myc-TB (Genoscreen), uses deep sequencing of a 24-plexed amplicon mix for simultaneous identification of mycobacterial species, genotyping, and prediction of drug resistance for MTBC. This includes 18 gene regions associated with resistance to 15 first and second-line drugs.[42–45] The ability to provide comprehensive detection of mutations and derive additional information from the MTBC genome before culture is available could have a dramatic impact on TB diagnosis and patient management. Approaches for the direct sequencing of sputum are currently slower than rapid methods such as pyrosequencing for detecting resistance, but can assess susceptibility to a wider range of drugs and concurrently provide relatedness among MTBC strains, supporting infection control efforts to

understand transmission. In fact, in March of 2024, the WHO issued recommendations for the use of tNGS to predict resistance to FQ as well as rifampin, isoniazid, pyrazinamide and ethambutol in respiratory specimens from people with confirmed pulmonary TB.[46]

There is growing evidence that sequencing of all mutations with a method such as WGS offers advantages over line-probe assays and other commercial molecular assays.[33,34,47] WGS-based DST can be accomplished for additional and even novel drugs at no extra cost, contingent only on the knowledge of characterized mutations, and this testing data could be helpful when designing new treatment regimens.[34] Shea et. al. developed a WGS assay to predict drug resistant TB (DR TB) for eight drugs and drug classes that, overall, demonstrates strong correlation with growth-based DST. Using this method with 128 isolates, prediction of FQ resistance was achieved with 100% accuracy.[33] While there is value in detecting all mutations as this can result in a higher sensitivity and provide more definitive data on which TB drugs to utilize, WGS methods typically require cultured material and can take many days to test and report findings compared to currently available tNGS based methods. Regardless, reports of shorter turnaround-times compared to growth-based DST with results available seven to eight days from a positive culture have also been described with the implementation of this testing.[33,48,49] A commercially available RUO assay, the QIAseq xHYB *Mycobacterium tuberculosis* Panel (Qiagen), utilizes whole genome enrichment to enable whole genome sequencing from lower quality samples, such as primary specimens.[27]

Table 1. Growth-based Drug Susceptibility Methods

Growth-based Method	Critical Concentration or MIC (for Sensititre)			Regulatory Status (US)
	MFX	LVX	OFX	
Agar Proportion 7H10 The number of colony forming units (CFU) growing on medium containing the drug at the critical concentration, as indicated, compared with the number of CFU growing on drug-free medium	0.5 µg/mL	1.0 ug/mL	2.0 ug/mL	LDT
Agar Proportion 7H11 The number of CFU growing on medium containing the drug at the critical concentra-tion as indicated compared with the number of CFU growing on drug-free medium	0.5 µg/mL	Not applicable	2.0 µg/mL	LDT
BACTEC™ MGIT™ 960 Drug Kit Growth in the presence of drug at the critical concentrations indicated	0.25 µg/mL	1.0[a] µg/mL	2.0 µg/mL	LDT
Sensititre™ *Mycobacterium tuberculosis* MYCOTB AST Plate Growth over a range of concentrations	Tested over range 0.06–8 µg/mL	Not applicable	Tested over range 0.25–32 µg/mL	RUO[b]

a WHO recommendation to lower critical concentration to capture low-level resistance.[12]

b Assays are commercially available as RUO, but can be validated for clinical testing.

Table 2. Molecular-based Drug Susceptibility Methods

Molecular-based Method	Determination of Resistance	Regulatory Status (US)
MTBDR*sl* line probe assay	Detection of mutations associated with FQ resistance by lack of hybridization to wild-type sequence probes and/or hybridization to probes containing known mutations visualized on a test strip.	RUO[b]
Sanger sequencing	Detection of mutations in *gyrA* and *gyrB* genes.	LDT
tNGS	Detection of mutations in *gyrA* and *gyrB* genes and other genes associated with resistance to additional drugs depending on assay design.	LDT
AmpliSeq for Illumina TB Research Panel (tNGS)	Detection of mutations *gyrA* and seven other genes associated with resistance to additional drugs.	RUO[b]
Deeplex® Myc-TB by Illumina and Genoscreen (tNGS)	Detection of mutations *gyrA* and *gyrB* and 16 other genes associated with resistance to additional drugs.	RUO[b]
AmPORE TB by Oxford Nanopore (tNGS)	Detection of mutations *gyrA* and *gyrB* and 22 other genes associated with resistance to additional drugs.	RUO[b]
QIAseq xHYB *Mycobacterium tuberculosis* Panel (WGS)	Detection of mutations in *gyrA* and *gyrB* genes while assessing the entire genome for other genetic predictors of drug-resistance.	RUO[b]
Whole-genome sequencing (WGS)	Detection of mutations in *gyrA* and *gyrB* genes while assessing the entire genome for other genetic predictors of drug-resistance.	LDT

Considerations for FQ DST

Growth-based DST

There is accumulating evidence that MFX may be useful in treating TB cases where FQ resistance is found at the critical concentration, but the MFX MIC is less than or equal to an established critical breakpoint (1.0 µg/mL in MGIT or 2 µg/mL in 7H10). Some studies suggest MIC testing when FQ resistance is detected[50] and indicate that testing at least two concentrations of MFX may differentiate low-level FQ resistance (still treatable with MFX or high dose LVX) from high-level FQ resistance (somewhat analogous to low and high-concentration Isoniazid (INH) testing).[50–53] WHO recommends that strains of MTBC with MIC levels above the critical concentration, but below or equal to the critical breakpoint, may be effectively treated with the higher daily dose of 800 mg MFX.[7] The same critical concentrations and critical breakpoints are recommended by WHO for agar proportion testing.[54] Thus, highlighting the importance of providing this information to clinicians for their treatment decision-making.

Molecular DST

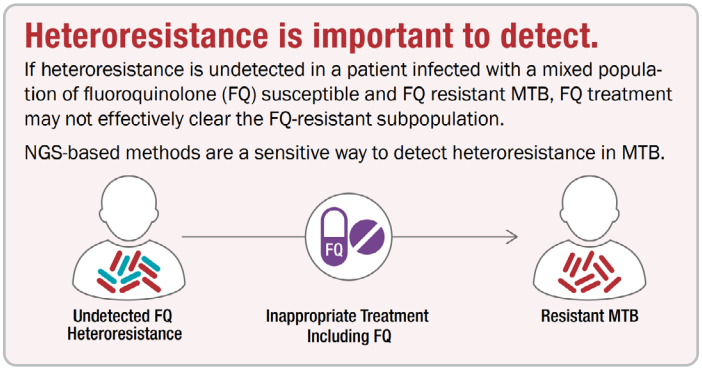
Although molecular DST methods are gaining favor among manufacturers, the US is still lacking in FDA-approved DST options for *M. tuberculosis.* The development and validation of laboratory developed molecular DST methods, particularly sequencing-based methods, requires specialized laboratories, rigorous method validation and extensive expertise.[32,33,55] Wet bench and dry bench (bioinformatic pipeline) methods must both be developed and validated and depending on the laboratory workflow, laboratories may also have to ensure that mycobacterial products brought outside of the biosafety level 3 (BSL-3) laboratory do not contain live organism.

Comparability Between Drugs Cross Resistance

The WHO Catalogue outlines that mutations in *gyrA* and *gyrB* confer cross-resistance to LFX and MFX, but states that the level of cross-resistance has not been fully elucidated.[15,16] However, certain mutations in the *gyrA* QRDR (*gyrA* Gly88Cys, Asp94Asn, Asp94Gly, Asp94His and Asp94Try) confer higher MFX MICs and more concordance between FQs. Additionally, *in vitro* data support variability based on the generation of drug and the effectiveness of LVX, MFX, and GFX in isolates determined to be OFX-resistant.[15,16,19] Therefore, it is important for clinicians and laboratorians to maintain a good working relationship so that the most appropriate drugs are tested, and effective patient care decisions can be made.

Heteroresistance

The presence of both FQ-susceptible and resistant subpopulations within specimens is an occurrence that may confound DST interpretation as DST methods vary in their ability to detect such heteroresistance. Moreover, identification of heteroresistance is important because when it goes undetected, inappropriate FQ treatment may select for the FQ-resistant sub-population.[56,57] In countries with a high burden of MDR-TB, literature describes FQ-heteroresistance frequencies ranging from 14–38%.[58] The ability to detect heteroresistance by growth-based methods is dependent upon the mutation frequency within a population and whether the mutation negatively impacts growth during primary culture. If mutation frequency is low and causes slower growth, a susceptible phenotype may be observed that does not truly mirror the heteroresistant population.[59]



Graphic from APHL's "TB: Next Generation Sequencing and Molecular Drug Susceptibility Testing" fact sheet.

If FQ treatment outcome is poor, additional testing utilizing DNA sequencing techniques should be considered. Literature has shown with experimentally-mixed populations that deep sequencing methods are more sensitive in the detection of heteroresistance compared to growth-based methods, and substantially more sensitive compared to Sanger sequencing.[58] Consequently, observed discordance between growth-based and molecular methods may be attributed to heteroresistance. While sequencing the MTBC genome to sufficient depth can detect heteroresistance, the absence of a mutation by molecular-based DST for *gyrA* or *gyrB* does not necessarily indicate susceptibility of the isolate, and these results must be considered in conjunction with other tests.[60]

Quality Assurance

CLIA certified laboratories must participate in a CLIA-approved proficiency testing (PT) program to satisfy regulatory requirements for DST performance. In the absence of a Centers for Medicare and Medicaid Services-approved PT program, laboratories can conduct alternative assessments that fulfil the requirements defined by CLIA. In the US, the College of American Pathologists provides PT for FQs and other anti-TB drugs; however, their program provides only two challenge isolates per year and includes only pan-susceptible strains. CDC offers the Model Performance Evaluation Program (MPEP) for MTBC DST twice a year, which is comprised of five isolates per panel that may be pan-susceptible or resistant to various drugs and provides growth-based as well as molecular-based results for each testing panel. Although the CDC program is not a formal, graded PT program, it offers a more comprehensive and diverse self-assessment tool for laboratories performing MTBC DST and provides an opportunity to compare results to those obtained by other participants using the same methods.

Impact on Clinical Outcomes

The Changing Role of FQs in TB Treatment

FQs have become increasingly important for treatment of both drug susceptible and drug resistant TB.[2–6] CDC's interim recommendations for use of the four-month all-oral treatment regimen for drug susceptible pulmonary TB in adults (older than 12 years) were based on results of a randomized clinical trial that compared the standard six-month treatment regimen with a shorter regimen that consisted of daily INH, PZA, MFX and high-dose rifapentine for eight weeks, followed by nine weeks of daily INH, MFX and high-dose rifapentine.[3] Before initiating this regimen, rapid baseline molecular DST and growth-based DST are recommended, to include testing for INH, PZA, FQ (MFX) and rifampin (proxy for rifapentine). Follow-up susceptibility testing may also be required to assess slow or non-responsiveness to treatment (e.g., if sputum cultures, collected at least monthly to culture conversion, remain positive at eight weeks).[4] The availability of shorter oral regimens will enable patients to be cured faster, with the potential to reduce treatment costs, improve patient quality of life and increase completion of therapy which will decrease the development of drug resistance.

Due to the propensity of FQ treatment to result in FQ-resistance,[61,62] it will be essential to determine levels of baseline resistance in various populations and monitor for the emergence of resistance to FQs in MTBC as usage increases. Moreover, it is not uncommon for a person with TB to be initially misdiagnosed with community-acquired pneumonia and treated with FQs prior to their TB diagnosis. For these patients, FQ mono-therapy may lead to initial clinical improvement, but it also increases the risk for development of FQ-resistant TB.[61] In fact, patients with prior exposure to FQ are more likely than non-exposed patients to have MFX-resistant isolates [14/22 (63.6%) vs. 8/25 (32.0%); P = 0.030].[62] Monitoring for the emergence of FQ resistance may best be accomplished by NGS methods.

Testing multiple FQs to improve patient outcomes may be needed, depending on the patient population served by the laboratory and needs of the treating clinician. For example, clinicians may increase dosage of treatment to overcome low-level resistance observed with some FQs, primarily MFX[63–66] and occasionally LFX. In this situation, knowing MFX and LVX susceptibilities (MICs) would be important for the clinician in making a treatment decision.

Areas of Ongoing Research

Comparison of Molecular vs. Growth-based DST

The WHO Catalogue represents a step forward in our ability to interpret NGS data in a clinically relevant and standardized way.[15,16] However, based on the second edition of the mutation catalogue, the sensitivity of WGS for predicting resistance is limited to 84.8% for MFX and 85.7% for LFX because the molecular basis of FQ resistance is yet to be fully elucidated.[16] Studies that utilize new methods to evaluate the comparability of molecular and growth-based DST are underway and may help to better delineate not only the mutations that correspond to FQ resistance, but also the level of resistance conferred by each mutation.[67] Continued investigation of the relationship between various mutations, MICs and clinical outcome is essential.[68]

Mechanisms of Resistance

While the *gyrA* mutations that confer resistance have been well defined, the roles of *gyrB,* efflux pumps and other mechanisms, including changes in outer membrane proteins,[12,69] require further investigation. Energy-dependent efflux pumps may reduce accumulation of FQs within the cell and enable low-level resistance and persistence of viable bacilli in large lesions where drug penetration is poor. Mutations leading to high-level FQ resistance could develop in these populations of persisting bacilli. Malik et al. study suggests further research into the role of *gyrB* mutations in FQ resistance.[69]

Sequencing and Bioinformatic Methodologies

As NGS technologies continue to become more accessible and in light of WHO's 2024 recommendations for the use of tNGS to predict resistance to FQ and other first-line drugs,[70] laboratories will increasingly add molecular DST that focuses on tNGS or WGS based methods. Ideally, manufacturers of diagnostic tests would focus on development and FDA clearance of molecular DST assays. Moreover, it will be essential for diagnostic manufacturers to monitor test performance as our understanding of the molecular basis for resistance continues to evolve.

This need for monitoring also applies to software tools designed to automate the analysis of WGS data. For example, three of the current tools (KvarQ, Mykrobe Predictor TB and TB Profiler) misclassified strain BTB-08-045 with *gyrA* T80A+A90G as resistant to at least one FQ because the respective mutation catalogues of these tools list A90G as a resistance mutation, whereas the tools CASTB and PhyResSE correctly classified the strain.[71–75]

Metagenomics and Direct Sequencing on Primary Specimens

An advantage of tNGS is that it is commonly used with primary specimens, whereas WGS more typically requires DNA extracted from cultured material. However, several studies using WGS or metagenomic NGS have highlighted the potential of these technologies for detection of MTBC directly from clinical specimens.[76–78] In one WGS-based study, a 22x average depth of MTBC genome coverage was obtained from sputum specimens by combining preanalytical benzonase treatment (to remove competing DNA) and MTBC target enrichment using the Twist (Twist Bioscience, USA) target capture system for MTBC.[76] Qiagen now has a commercially available product, the QIAseq xHYB *Mycobacterium tuberculosis* Panel that uses hybrid capture probes tiled across the MTBC genome to target and enrich the whole genome and enable WGS directly from specimens.[27] Further utilization and refinement of such procedures would allow for efficient detection, DST and molecular surveillance of MTBC using a single analytical method and is of great interest.

Population-based Studies of FQ Resistance

While FQ may be considered first-line therapy for TB when newer short-course regimens are utilized, this concept is relatively recent. More commonly, first-line DST panels consist of the drugs associated with the traditional six- and nine-month RIPE treatment regimens (i.e., rifampin, isoniazid, pyrazinamide, ethambutol). Consequently, the frequency of FQ monoresistance may be underestimated, as FQ susceptibility testing is typically not performed when RIPE DST indicates susceptibility. In fact, FQ monoresistance is considered uncommon, with studies describing prevalence ranging from 0.15–4%.[8,12,61,79–83] Some studies suggest that prior exposure to FQs may lead to higher risk of developing FQ resistance.[8,83] One retrospective study found an association of MTBC FQ resistance in patients that had received more than 10 days of FQ therapy prior to MTBC diagnosis, with the highest risk for developing resistance occurring when MTBC diagnosis exceeded 60 days.[83]

Prior FQ exposure can occur during treatment of community-acquired pneumonia. There has been published concern regarding a lack of stated caution for utilization of FQ monotherapy in individuals with risk of MTBC infection within the treatment guidelines for community-acquired pneumonia.[61,80] In cases of MDR infections, additional FQ resistance alone was associated with poorer outcomes that seconded only extensively drug-resistant (XDR) infections.[12] This study also found that 75% of the cases of resistant strains (FQ, second line injectables or resistance to both) were from patients that had not previously received treatment with second line MTBC drugs—suggesting primary infection with resistant strains rather than selective pressure arising to resistant strains.[12] Further studies are needed to truly determine prevalence of monoresistant FQ strains and how this is distributed globally.

Critical Concentration and Breakpoints

Research challenging the current critical concentration testing protocols and the development of new testing breakpoints is ongoing.[84] Further determination of more accurate distribution of MICs in wild type MTBC and the relationship to clinical outcomes is needed.

Laboratory Guidance

Laboratories should consider the following when dealing with FQs:

- Use fresh cultures for preparation of test inoculum for growth-based DST.
- Ensure a standard inoculum for DST; laboratories may consider validating a method for inoculum preparation as recommended by WHO, EUCAST, CLSI or the test manufacturer.
- Monoresistant INH should be tested for susceptibility to second-line drugs if the clinician is planning to include FQ in the treatment regimen.
- For laboratories testing MFX, it is recommended to test two concentrations, including lower concentration to detect the presence of low-level resistance and indicate whether the organism is susceptible to FQ at lower concentrations. Testing a second, higher concentration is recommended by WHO when there is resistance at the lower concentration.
- Initial molecular or growth-based DST results indicating resistance should be reported immediately, prior to the availability of confirmatory results; the healthcare provider should be informed that confirmatory testing is being performed.
- Testing for mutations using molecular methods is potentially useful, as mutations associated with resistance may be rapidly identified. However, absence of mutations does not confirm susceptibility.
- Heteroresistance appears to be more common for FQs than for most other drugs. This can result in a discrepancy between growth-based results (detecting a minority resistant population) and molecular-based results (detecting a majority wild-type sequence).
- When *gyrA* sequences are the first result available for detection of FQ resistance, these are useful in predicting whether the organism has low- or high-level resistance. For this reason, when FQ resistance is suspected, laboratories should consider submitting an isolate or smear-positive specimen to a reference laboratory for DNA sequencing.
- When patients do not respond to therapy after three months, laboratories should follow APHL and CDC guidelines for Submission of Sputum Specimens for MTBC testing for re-evaluation of drug resistance. If your laboratory is unable to perform molecular testing for RIF, samples can be referred to a jurisdictional public health laboratory for testing and/or those laboratories can refer to the following services:
    - CDC Molecular Detection of Drug Resistance Service (MDDR) provides rapid algorithm-based testing and other comprehensive testing services at no cost to all public health laboratories.
    - The National Public Health Laboratory Drug Susceptibility Testing (DST) Reference Center for *Mycobacterium tuberculosis* provides rapid algorithm-based testing at no cost to enrolled public health laboratories. Enrollment is restricted to public health laboratories performing TB DST on fewer than 50 isolates/year.

Abbreviations

AP.....Agar proportion

BSL-3 .....Biosafety Level 3

CAP .....College of American Pathologists

CDC.....US Centers for Diseases Control and Prevention

CFU.....Colony forming units

CIP.....Ciprofloxacin

CLIA .....Clinical Laboratory Improvements Amendment

DR TB.....Drug resistant tuberculosis

DST .....Drug susceptibility testing

EUCAST ..European Committee on Antimicrobial Susceptibility Testing

FDA .....US Food and Drug Administration

FQ.....Fluoroquinolones

GFX .....Gatifloxacin

INH .....Isoniazid

LDT.....Laboratory developed test

LVX.....Levofloxacin

MDR .....Multidrug-resistant

MDR-TB ..Multidrug-resistant tuberculosis

MFX.....Moxifloxacin

MIC.....Minimum inhibitory concentration

MPEP.....Model Performance Evaluation Program

MTBC .....Mycobacterium tuberculosis complex

NGS.....Next generation sequencing

OFX .....Ofloxacin

PZA .....Pyrazinamide

QRDR.....Quinolone-resistance-determining region

RIPE.....Rifampin, isoniazid, pyrazinamide, ethambutol

RUO.....Research use only

TB.....Tuberculosis

tNGS .....Targeted next generation sequencing

WGS.....Whole genome sequencing

WHO .....World Health Organization

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