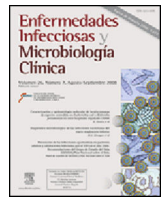




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Review article

Methods for determining the antimicrobial susceptibility of mycobacteria[☆]



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ABSTRACT

Mycobacteria are a large group of microorganisms, multiple species of which are major causes of morbidity and mortality, such as tuberculosis and leprosy. At present, the emergence and spread of multidrug-resistant strains of *Mycobacterium tuberculosis* complex are one of the most serious health problems worldwide. Furthermore, in contrast to *M. tuberculosis* and *Mycobacterium leprae*, non-tuberculous mycobacteria (NTM) are more frequently isolated and, in many cases, treatment is based on drug susceptibility testing. This article is a review of the different methods to determine the *in vitro* drug susceptibility of *M. tuberculosis* complex and the most relevant NTM isolates. The molecular techniques currently used for rapid detection of resistance of clinical specimens are also analysed.

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Métodos de determinación de sensibilidad a los antimicrobianos en micobacterias

RESUMEN

Las micobacterias son un amplio grupo de microorganismos en el que múltiples especies son causa de una importante morbimortalidad, como la tuberculosis y la lepra. La aparición y diseminación de cepas del complejo *Mycobacterium tuberculosis* resistentes a diversos fármacos constituye en la actualidad uno de los problemas sanitarios de mayor gravedad a nivel mundial. Por otro lado, las micobacterias diferentes de *M. tuberculosis* y *Mycobacterium leprae*, denominadas micobacterias no tuberculosas (MNT), son aislamientos cada vez más frecuentes, requiriendo en muchos casos un tratamiento que precisa una orientación sobre la sensibilidad de estos microorganismos a los antimicrobianos. En el presente artículo se revisan los métodos para determinar la sensibilidad *in vitro* a los antimicobacterianos de los aislamientos del complejo *M. tuberculosis* y las MNT más relevantes. Además, también se realiza un análisis de las técnicas moleculares de detección rápida de la resistencia a partir de las muestras clínicas.

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Introduction

Currently, more than 170 species of mycobacteria have been reported, and many of them are a major cause of morbidity and mortality in humans. Notable among them is tuberculosis (TB), caused by the *Mycobacterium tuberculosis*¹ complex. According to the WHO, around 9.6 million people became ill from TB in 2014 and 1.5 million died from it, with it being the primary cause of

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death by an infectious agent.¹ Also, in recent years, strains resistant to multiple drugs have appeared and are spreading. Thus, it is estimated that 480,000 people developed multidrug-resistant TB (MDR-TB; resistance to at least isoniazid and rifampicin) worldwide in 2014, of which 9% would have extended resistance (XDR-TB) to at least one of the second-line injectable drugs (capreomycin, kanamycin, or amikacin) and to a fluoroquinolone. Therefore, the rapid detection of resistance to antituberculosis drugs is fundamental to achieve adequate treatment and prevent the onset and spread of MDR-TB. Furthermore, nontuberculous mycobacteria (NTM) or environmental mycobacteria have become increasingly prevalent, with many of them being pathogenic (mycobacteriosis) and requiring a specific treatment that, in many cases, should be oriented by *in vitro* antimicrobial susceptibility testing.^{2–4}

Antimicrobials

Although the therapeutic arsenal for mycobacterial infections is very limited, some new drugs are currently available, although not all have the same activity or are used to treat the same types of infection or different species (Table 1).⁵

Drugs used against the *M. tuberculosis* complex

Drugs called “first line” treatments include those administered as the first choice and are isoniazid (H), rifampicin (R), ethambutol (E), pyrazinamide (Z), and, for historical reasons, also streptomycin (S).^{5–7} The first four are those recommended by the WHO for a 6-month regimen (2HREZ+4HR). In cases of resistance, allergy, intolerance, hepatic toxicity or interaction with one or more of these drugs, second-line drugs should be used, including rifamycins (rifabutin, rifapentine), quinolones (ofloxacin, levofloxacin, moxifloxacin), aminoglycosides (amikacin, tobramycin), capreomycin, cycloserine, linezolid, isoniazid analogues (ethionamide, prothionamide), clofazimine, PAS or thiacetazone.^{5–7} In cases of MDR-TB

or XDR-TB, recently developed drugs may also be used, such as bedaquiline and/or delamanid.⁸

Drugs used against nontuberculous mycobacteria

The treatment of NTM, with the exception of leprosy, is less standardised than that of TB, with wide variations between species.^{2,9} For most slow-growing species, the mainstays of treatment are clarithromycin, ethambutol and amikacin, complemented with other drugs such as quinolones, rifamycins, linezolid, and probably, with a lesser degree of efficacy, cephalosporins, carbapenems and tetracyclines. *Mycobacterium kansasii* and *Mycobacterium xenopi* infections are treated similarly to TB, except for pyrazinamide, since it does not have activity against NTM.^{2,9} Rapidly growing species are resistant to classic antituberculosis drugs and also have clarithromycin as the mainstay of treatment, with the exception of *Mycobacterium fortuitum*, which is resistant, as well as 80–85% of *Mycobacterium abscessus* isolates.^{2,9}

Drug susceptibility testing in *M. tuberculosis* complex

The history of susceptibility testing for the *M. tuberculosis* complex arose parallel to the development of specific pharmacological treatment for TB, which began with the discovery of streptomycin in 1944. From the beginning, it was observed that monotherapy achieved an initial clinical improvement followed by a relapse of the disease. This phenomenon, known as ‘fall and rise’, was due to the selection of resistant bacteria. In 2000, the WHO defined resistance in various categories from an epidemiological point of view: (a) *Resistance in new cases*: strains isolated in patients who have never received antituberculosis treatment for more than one month. (b) *Resistance in treated cases*: strains isolated in patients who have previously received antituberculosis treatment for more than one month. (c) *Multidrug resistance* (MDR-TB): joint resistance to at least isoniazid and rifampicin. (d) *Poly-resistance*: resistance to more than one drug not including isoniazid and rifampicin at

Table 1
Mechanism and action spectrum of antimycobacterials.

Mechanism of action	Drug	Action spectrum	Population–action
Inhibition of the wall synthesis	Isoniazid (isonicotinic acid hydrazide)	MTC, MK, MX	Bactericide (active growth), bacteriostatic (latent)
	Ethionamide, prothionamide and thiacetazone	MTC, variable in NTM (MK and <i>M. marinum</i>)	Bacteriostatics
	Ethambutol	MTC, various slow-growing NTM	Bacteriostatic
	Pyrazinamide	MTC, with the exception of <i>M. bovis</i>	Bactericide (active and latent growth)
	Cycloserine	MTC (use restricted to MDR), variable in NTM	Bacteriostatic
Inhibition of protein synthesis	Delamanid	Use restricted to MDR	Bacteriostatic
	Rifamycins (rifampicin, rifabutin, rifapentine)	MTC, MK, MX, 50% in MAC, variable in the rest of species	Bactericides in active and latent growth
	Aminoglycosides (streptomycin, amikacin, tobramycin)	MTC and most NTM (especially amikacin)	Bactericides
	Linezolid	MTC (use in MDR). Variable for NTM	Bactericide
	Capreomycin	MTC	Bactericide in active growth
Inhibits folic acid synthesis	Macrolides (clarithromycin, azithromycin)	Very effective in MAC. Active in NTM, except for <i>M. fortuitum</i> and <i>M. abscessus</i> . Of little use in MTC	Bacteriostatics
	Tetracyclines and tigecycline	Variable in NTM. Tigecycline more effective	Bacteriostatics
	Dapsone	<i>M. leprae</i>	
Inhibits DNA synthesis	PAS (para-aminosalicylic acid)	MTC (use in MDR)	Bacteriostatic
	Co-trimoxazole	Variable in NTM	Bacteriostatic
Interferes in Fe metabolism	Fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin)	MTC, NTM (MAC group 50%, rest variable)	Bactericides in active growth
	Clofazimine	MTC, variable in NTM	Bactericide
Inhibits ATP synthase	Bedaquiline	Use restricted to MDR	Bacteriostatic

MAC, *M. avium* complex; MDR, multidrug-resistant tuberculosis; MK, *M. kansasii*; NTM, nontuberculous mycobacteria; MTC, *M. tuberculosis* complex; MX, *M. xenopi*.

the same time. (e) *Combined resistance*: sum of all resistance types in a specific area. This indicates the resistant strain load in a community. In 2006, the category of *Extended resistance* (XDR-TB) was added, which was mentioned in the introduction.

The definitive confirmation of *M. tuberculosis* complex drug resistance must always be conducted using microbiological methods. In studies in the 1960s by Canetti et al.,¹⁰ the frequency of spontaneous mutations associated with each drug was established. In the case of isoniazid, ethambutol and streptomycin, a resistant mutant appeared in one out of 10^{-5} – 10^{-7} bacteria, for rifampicin in one out of 10^{-7} – 10^{-9} and for pyrazinamide in one out of 10^{-2} – 10^{-4} . The theoretical frequency of mutants resistant to more than one drug would be the exponential sum of the individual mutants resistant to each drug. The bacterial populations present in the lesions were also estimated. Thus, small infiltrates had populations of 10^3 – 10^5 bacteria, and cavities could reach 10^7 – 10^9 bacteria which makes it very improbable that simultaneous mutations to more than one drug would occur spontaneously. TB is deemed resistant when 1% or more of the bacterial population is resistant to a certain drug.¹⁰

The *critical concentrations* of the different drugs were established by international consensus and represent the lowest concentrations that inhibit the growth of “wild strains” (susceptible strains) of the *M. tuberculosis* complex that have never been exposed to said drugs, while not inhibiting the strains from patients that do not respond to treatment, considered resistant. They were initially postulated for the Löwenstein–Jensen medium, and later equivalencies were established for Middlebrook 7H10 and 7H11 agar media, as well as for automated liquid media culture systems.

In vitro susceptibility testing in *M. tuberculosis* complex must be performed on the first isolate obtained from a new patient, in cases of suspected therapeutic failure and before the disease relapses.^{7,11–13} Also, when the TB simultaneously affects more than one organ, the tests must be done individually for each location, without assuming that the results will always coincide. All first-line drugs should be included (isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin), since they provide information about the therapeutic scheme currently recommended for most patients.^{7,11–14} Traditional second-line drugs (amikacin, capreomycin, clofazimine, cycloserine, ethionamide, kanamycin and PAS) and new ones, such as fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), linezolid, and, in certain cases, rifabutin, must be evaluated whenever: (a) a strain is resistant to isoniazid, rifampicin, ethambutol or pyrazinamide; (b) when mutations in genes that encode resistance to isoniazid and/or rifampicin are detected using molecular techniques; and (c) when clinical-epidemiological information is available that recommends doing so.^{13–15}

Solid culture media-based methods

The *absolute concentrations* method, described by Meissner, is based on the comparison of the number of colonies in the presence of the drug to the number of colonies in the culture medium without the drug. The results are affected by the size of the inoculum and by the precision in the definition of the critical concentration of each drug. The *resistance ratio* method, described by Mitchison, compares the minimum inhibitory concentration (MIC) of a specific strain to that of a reference strain. The results depend on the adequate standardisation of the drugs and the inoculum, although the critical concentrations do not need to be defined. The *multiple proportion* method, described by Canetti et al.¹⁰ in 1963, shows the proportion of resistant bacilli present in a culture. Micro-organism resistance is considered clinically significant when the resistant bacterial population exceeds 1%. This is the most precise method and the one that produces fewest false results, which is why it is

the most widely used worldwide.^{7,11,13,16} The *epsilometer* (E-test) method is based on a gradient of antimicrobial diffusion combining the principles of disc diffusion and agar dilution in the study of *in vitro* susceptibility. It is a quantitative technique to determine the MIC of antimicrobials.¹⁷

Liquid culture media-based methods

There are currently two nonradiometric systems for susceptibility testing: BACTEC™ MGIT™ 960 (Becton–Dickinson Diagnostics, Sparks, MD, USA) and VersaTREK® (Thermo Scientific, Westlake, OH, USA).^{7,11,13,18} Both use supplemented Middlebrook 7H9 medium, and the growth is shown *via* detection of the consumption/production of certain gases. The initial and secondary critical concentrations that are analysed come predetermined by the manufacturer for first-line drugs. Although the detection of MDR-TB does not usually pose any problems, there are some inter-laboratory discrepancies with streptomycin, ethambutol and pyrazinamide. In the case of pyrazinamide, since it is active in acidic media (pH 5.5), the pH of the culture medium must match these conditions, despite the fact that approximately 10% of *M. tuberculosis* isolates do not grow well at this pH. On the other hand, it is also possible to analyse critical concentrations of other second-line drugs if the assessed substance is available.

Alternative susceptibility methods

In *mycobacteriophage*-based techniques, when adding the phages to a culture in the presence of an antibiotic, the phages will only infect the viable mycobacteria, which are the resistant ones. These have been applied in isoniazid and rifampicin susceptibility tests, but they present problems with susceptibility, specificity and a high rate of contamination. *Flow cytometry*, using cell permeable nucleic acid stains, shows viable mycobacteria (the control mycobacteria and the drug-resistant ones) with higher levels of fluorescence compared to the non-viable ones (drug-susceptible). One of the drawbacks is the high cost of the equipment. *Broth microdilution* (Mycob Sensititre® Thermo Scientific) allows for the MIC of multiple drugs to be determined. The greatest difficulty lies in reading and interpreting the results, since the growth of these bacteria is not homogeneous. The *nitrate reductase test* is based on the capacity of the *M. tuberculosis* complex to reduce nitrates to nitrites. Tubes of Löwenstein–Jensen medium incorporating KNO₃, with and without drugs, are used for this purpose. *Microscopic-observation drug-susceptibility* (MODS) is based on the early detection of the cord factor exhibited by the *M. tuberculosis* complex, with the help of an inverted microscope. *Resazurin microtiter assay* (REMA) is an indirect method based on the detection of colour changes in the culture medium due to oxidation (blue) and reduction (pink) of resazurin.

The WHO considers that nitrite reductase, MODS and REMA are simple, cheap techniques that can be used for rapid screening for resistance to isoniazid and rifampicin in countries with limited resources.^{11,13,19} Susceptibility and specificity data exceed 95%.

Antimicrobial susceptibility testing in nontuberculous mycobacteria

Although more than 160 species of NTM have been reported, susceptibility testing is only recommended in clinically-significant isolates for which a correlation has been shown between *in vitro* and *in vivo* results.^{2,13} Thus, the Clinical and Laboratory Standards Institute (CLSI) establishes recommendations only for the group comprising *Mycobacterium avium* complex, *M. kansasii*,

Mycobacterium marinum and non-pigmented rapidly growing mycobacteria.⁷

Susceptibility testing in slow-growing nontuberculous mycobacteria

In general, the methodology of choice that CLSI recommends is microdilution in cation-adjusted Mueller Hinton (CAMH) broth, supplemented with OADC or ADC. However, in some cases there are alternatives to this methodology.

Recommendations for *M. avium* complex

The treatment of *M. avium* complex (MAC) is based on the use of clarithromycin as an essential or primary drug, and ethambutol and rifampicin (or rifabutin) as adjunct therapies, although there are data showing that other antibiotics (amikacin, linezolid and moxifloxacin) could be useful in some cases. Given that a clinical correlation has only been demonstrated with *in vitro* tests for macrolides, the CLSI recommends that only these drugs be tested.⁷ However, when resistance to clarithromycin is present, it is reasonable to test other drugs (moxifloxacin, linezolid and aminoglycosides). Clarithromycin's activity is very sensitive to the culture medium and its pH, therefore the MICs used generally as cut-off points correspond to a CAMH medium at a slightly basic pH of 7.3–7.4. Another method that can be used is microdilution using the BACTEC™, MGIT™ 960 and VersaTREK® systems.^{2,13} There is currently a commercial microdilution technique in microtitration plates (Slomyco, Sensititre®, Thermo Scientific), that allows for simple, reproducible *in vitro* susceptibility testing with a standard incubation of 7 days. Although it includes 13 antimicrobials with multiple concentrations, it is important to maintain the previously stated interpretation criteria (Table 2).

Recommendations for *M. kansasii*

Standard treatments for infections caused by this mycobacteria include rifampicin (or rifabutin), isoniazid, and a third drug (ethambutol or streptomycin) for 18 months (at least 12 months of negative cultures). In cases of possible resistance, susceptibility should be conducted *in vitro* for these and other potentially-active antimicrobials (clarithromycin, moxifloxacin or levofloxacin, amikacin, linezolid and rifabutin).^{2,7,13} One aspect to highlight is that *M. kansasii* naturally presents a somewhat decreased susceptibility to isoniazid in comparison with *M. tuberculosis*, although it has clinical activity.

The recommended method is microdilution, although the proportion method in Middlebrook 7H10 agar and automated

nonradiometric systems have been used. As mentioned previously in MAC, there is currently a commercial microdilution technique that, although it includes multiple antimicrobials with diverse concentrations, it is important to follow the current interpretation criteria (CLSI).

Recommendations for *M. marinum*

Antimicrobial susceptibility testing is currently only recommended for this species when there is a clinical and microbiological failure, using the broth microdilution method at 28–30 °C for 7 days.^{2,7,13}

Other slow-growing nontuberculous mycobacteria

Generally, it is recommended to perform *in vitro* susceptibility testing using broth microdilution with the same drugs and the same concentrations as for *M. kansasii* for all species with good growth (non-deficient) and in those in which therapeutic failure and persistent positive cultures are detected.

Susceptibility testing in rapid-growing nontuberculous mycobacteria

Treatment for these micro-organisms is usually conducted with drugs against which the remaining species are resistant or, at least, have a lesser susceptibility. Due to all of this, *in vitro* susceptibility tests have certain differentiating features from the rest of the mycobacteria. The recommended technique is broth microdilution with an incubation of 3–4 days at 30 °C (Table 3).^{2,3,7,13} Other methods present interpretation problems and should not be used. We must keep in mind the possible presence of inducible *erm* methylases. Therefore, reincubating the plates for up to 14 days and assessing the increase of the macrolides' MIC is recommended to detect this phenomenon. There is currently a commercial microdilution technique designed specifically for rapid-growing mycobacteria and aerobic actinomycetes (Rapmyco, Sensititre®, Thermo Scientific).

Mycobacteria resistance mechanisms

Antibacterial resistance is mainly due to chromosomal mutations in genes that encode their target or the activating enzymes. They can be spontaneous or drug-induced after exposure to monotherapy.^{2,3,20,21}

Table 2

Interpretation criteria for susceptibility testing of the *M. avium* complex via broth microdilution.

Antimicrobial	MIC (µg/ml)		
	Susceptible	Intermediate	Resistant
Amikacin ^a	≤32		≥64
Clarithromycin			
Microdilution (pH 7.3–7.4) ^b	≤8	16	≥32
Radiometric (pH 7.3–7.4) ^c	≤4	8–16	≥32
Radiometric (pH 6.8) ^c	≤16	32	≥64
Moxifloxacin	≤1	2	≥4
Linezolid	≤8	16	≥32

^a The MIC for amikacin is not defined. Due to assimilation into other species, it may be considered resistant >32 µg/ml.

^b Cation-adjusted Mueller Hinton (CAMH) medium, supplemented with OADC or ADC.

^c 12B radiometric medium. Some authors consider the same MICs for the BACTEC MGIT 960 and VersaTREK media.

Table 3

Interpretation criteria for susceptibility testing of rapid-growing mycobacteria via broth microdilution.

Antimicrobial	MIC (µg/ml)		
	Susceptible	Intermediate	Resistant
Amikacin ^a	≤16	32	≥64
Cefoxitin	≤16	32–64	≥128
Ciprofloxacin/levofloxacin	≤1	2	≥4
Moxifloxacin	≤1	2	≥4
Clarithromycin	≤2	4	≥8
Doxycycline/minocycline	≤1	2–8	≥16
Imipenem	≤4	8	≥16
Co-trimoxazole ^b	≤2/38	–	≥4/76
Tobramycin ^c	≤4	8	≥16
Linezolid	≤8	16	≥32

^a If the isolate is a strain of *M. abscessus* and the MIC is ≥64 µg/ml, it must be repeated to confirm.

^b MIC defined by an 80% inhibition in growth control.

^c Only report in *M. chelonae*.

Isoniazid

Resistance is associated above all with mutations in 2 genes (68–90%): (a) the *katG* gene, that is responsible for activating the drug; the most common mutation affects codon 315 and is related to high-level resistance (60–70% of resistance), and (b) the *inhA* gene, which intervenes in the synthesis of the bacterial wall. Mutations (8–20%) cause low-level and cross-resistance with ethionamide.^{13,20,21} NTM, except for *M. kansasii* and *M. xenopi* are resistant due to incapacity of activation of the drug.^{2,3,13}

Rifampicin and other rifamycins

Resistance to the *M. tuberculosis* complex is related to mutations in the *rpoB* gene (>95%), involved in protein synthesis. Codons 526 and 531 are the most affected and confer high-level and cross-resistance with other rifamycins.^{13,20,21} Mutations in some codons will maintain susceptibility to rifabutin. Similar mutations in NTM have been described.

Aminoglycosides and capreomycin

Resistance is based on ribosomal mutations involved in protein synthesis. Resistance to streptomycin is associated with mutations (50–60%) in the *rpsL* and *rrs* genes (codons 530–912), while resistance to amikacin, kanamycin, and capreomycin is associated with mutations in the *rrs* gene (codons 1400–1500).^{13,20,21} There is partial cross-resistance between them, with isolated resistance to streptomycin that may be susceptible to the others. The *tlyA* gene has been linked to resistance to capreomycin and the *eis* gene has been linked to resistance to kanamycin.

Ethambutol

Resistance is related to mutations in the *embB* gene, which is involved in bacterial wall synthesis. The majority (60–80%) have been described in codons 306 and 406.^{13,20,21} Some species of NTM have natural resistance.^{2,3}

Pyrazinamide

Resistance is associated with mutations throughout the *pncA* gene (72–97%) that regulates drug activation. *Mycobacterium bovis* and its BGC variant, as well as NTM, have natural resistance.^{2,3,13,20,21}

Fluoroquinolones

The most frequent mechanism is mutations in the *gyrA* gene (85%) and the *gyrB* gene (10%). There is cross-resistance between fluoroquinolones, but not full resistance, depending on the affected codons.^{2,3,13,20,21}

Macrolides and ketolides

Resistance is linked to mutations in the 23S ribosomal gene and, above all, to inducible methylases regulated by the *erm* gene. These are observed in species such as *M. fortuitum* and *M. abscessus* subsp. *abscessus* and subsp. *bolletii*.²

Beta-lactams

Resistance is associated with a decrease in the permeability and affinity of the PBPs and, mainly, to beta-lactamases.^{2,3}

Tetracyclines and glycylglycines

Resistance to tetracyclines is due to ribosomal protection proteins or efflux pumps.^{2,3} To date, resistance to tigecycline has not been demonstrated.

Oxazolidinones

The resistance of mycobacteria to linezolid is associated with mutations in the rRNA 23S gene.^{13,20}

Co-trimoxazole

The resistance mechanism is not clear in mycobacteria.

Bedaquiline and delamanid

The resistance to bedaquiline has been associated with a mutation in the *mmpR* gene and the resistance to delamanid has been associated with the *fbiA* and *fgd1* genes.²²

Molecular detection of resistances

The reference method for determining resistance to antituberculosis drugs is the conventional phenotypic susceptibility test, although the results tend to take several weeks.^{7,11,13} Molecular detection of resistance, based on showing chromosomal mutations, offers quick and useful results.^{21,23} Commercial techniques are those which are most widely used due to their greater standardisation (Table 4). The most used are Xpert[®] MTB/RIF Assay (Cepheid, Sunnyvale, CA, USA) and GenoType[®] MTBDRplus (Hain Lifescience, Nehren, Germany), both approved by the WHO for the rapid diagnosis of MDR-TB. Xpert[®] MTB/RIF allows for diagnosis of the *M. tuberculosis* complex and the detection of mutations resistant to rifampicin in <2 h, with minimal manipulation, limited to inserting the sample into a cartridge where the entire amplification and detection process is performed. The system uses five fluorescent probes covering the natural sequence of the 81 pb region of the *rpoB* gene where more than 95% of resistances to rifampicin occur. For the diagnosis of pulmonary and extrapulmonary TB, susceptibility is 95–98% in samples with positive microscopy and 67–69% with negative microscopy, taking the culture as reference.²⁴ The specificity is 99%. In synthesis, Xpert MTB/RIF[®] provides 23% more positive cases than microscopy. Its greatest drawbacks are that it only detects resistance to rifampicin and its financial cost, although for countries with limited resources, the cost is 6–7 times lower (less than €10).²⁴ The GenoType[®] MTBDRplus is based on amplification and reverse hybridisation on nitrocellulose strips that contain immobilised probes corresponding to natural sequences and sequences of the most frequent mutations in the *rpoB* (rifampicin) and *katG* and *inhA* (isoniazid) genes. Currently, it can be used based on cultures or clinical samples, even with negative bacilloscopy. Various meta-analyses establish a global susceptibility of 98% for resistance to rifampicin and 84.3% for isoniazid, with 98–99.5% specificity. Samples with negative microscopy can have 5–12% invalid results due to low susceptibility. Results that are not interpretable due to the simultaneous absence of natural and mutated bands in the same *locus*, due to mutations not contained in the kit have been reported. Another possibility is a discrepant result with the sensitive genotype and resistant phenotype, due to mechanisms other than those detected by the commercial product. The resistant genotype and sensitive phenotype option would be caused by the coexistence of mixed or heteroresistant populations or low-level resistances, that have been described in 4–13% of cases.²⁵ At least 50% of patients with MDR-TB are resistant to other drugs. Tests have also been marketed to detect mutations

Table 4

Marketed methods for the detection of resistant mutations to antituberculosis drugs.

Test	Brand	Method	Detection	Year
TB Resistance INH/RIF	AID	PCR, LPA	H, R	2013
TB Resistance FLQ	AID	PCR, LPA	FQ	2013
TB Resistance AMG	AID	PCR, LPA	AG	2013
BioFilmChip MDR TB	Autogenomics	PCR, microarray	H, R	2011
Drug Resistance Detection Kit	CapitalBio Co	PCR, microarray	MDR	2009
Xpert MTB/RIF assay	Cepheid	RT-PCR	R	2009
NTM/MDR TB	NIPRO Co	PCR, LPA	H, R	2012
INH	NIPRO Co	PCR, LPA	H	2012
PZA	NIPRO Co	PCR, LPA	PZ	2012
FLQ	NIPRO Co	PCR, LPA	FQ	2012
Anyplex II MTB/MDR/XDR	Seegene	RT-PCR	H, R, FQ, AG	2012
Anyplex MTB/MDR/XDR	Seegene	RT-PCR	H, R	2012
VereMTB Detection	Veredus Labs	PCR, microarray	H, R	2012
MeltPro RIF	Zeesan Biotech	RT-PCR	R	2014
MeltPro INH	Zeesan Biotech	RT-PCR	H	2014
MeltPro FLQ	Zeesan Biotech	RT-PCR	FQ	2014
MeltPro AMG	Zeesan Biotech	RT-PCR	AG	2014
Genedrive Mycobact iD	Epistem	RT-PCR	R	2013
INNO-LiPA Rif.TB	INNO Genetics/Fujirebio	PCR, LPA	R	N/A
GenoType MTBDRplus v2.0	Hain Lifescience	PCR, LPA	H, R	2012
GenoType MTBDRsl v1.0	Hain Lifescience	PCR, LPA	AG, FQ, E	2009
GenoType MTBDRsl v2.0	Hain Lifescience	PCR, LPA	AG, FQ	2013
iC2.0-MYC assay	iCubate	PCR, microarray	H, R, E, FQ, AG, BD	2012
HYDRA	Insilixa Inc.	PCR, microarray	H, R, PZ, E, FQ, AG	2015

AG, aminoglycosides; BD, bedaquiline; E, ethambutol; FQ, fluoroquinolones; H, isoniazid; LPA, line probe assay; N/A, not available; PCR, polymerase chain reaction; PZ, pyrazinamide; R, rifampicin; RT-PCR, real-time PCR.

Modified from *Tuberculosis Diagnostics Technology and Market Landscape*, UNITAID WHO 2015.

of resistance to second-line drugs. The most well-known product is GenoType® MTBDRsl (Hain Lifescience). Two versions exist: v1.0 and v2.0. The first is directed at mutations of resistance to capreomycin and the aminoglycosides amikacin and kanamycin in the *rrs* gene (codons 1401 and 1484), as well as to fluoroquinolones in the *gyrA* gene (codons 90, 91 and 94), and to ethambutol in the *embB* gene (codon 306). A recent analysis observed good susceptibility to fluoroquinolones (83–85%), but lower susceptibility to aminoglycosides (77%), attributable to kanamycin (69.9%).²⁶ Version v2.0 seeks to improve yield including the *gyrB* gene for fluoroquinolones and the *eis* gene for kanamycin, suppressing the ethambutol of the kit. It has recently been evaluated with a susceptibility of 96% to kanamycin. However, a decrease in specificity for injectables (91%) has also been observed, due to the detection of mutations of still unknown significance in the *eis* gene in some susceptible strains.²⁷ Aside from the above, the following products are also being distributed to a certain extent: INNO-LiPA® RifTB (INNO Genetics, Gent, Belgium/Fujirebio, Tokyo, Japan), Anyplex II™ MTB/MDR/XDR (Seegene, Seoul, Korea) and the TB Resistance® Assay (AID; Autoimmun Diagnostika, Strassberg, Germany).^{18,21,23} Although not discounting phenotypic susceptibility tests, the main use of molecular resistance detection methods, in our environment, is to quickly confirm or rule out, and to a greater or lesser extent, based on the drugs, resistance in cases of high suspicion. Recently, a method has been marketed (GenoType® NTM-DR, Hain Lifescience) that, in addition to identifying some NTM species, allows detection of the *erm* gene in *M. abscessus*, and of the mutations in the *rrs* (aminoglycosides) and/or *rrl* (macrolides) genes.

Conflicts of interest

The authors have declared that they have no conflicts of interest.

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