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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.

Métodos de determinación de sensibilidad a los antimicrobianos en micobacterias

Palabras clave:
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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.

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 *M. marinum*)	Bacteriostatics
	Ethambutol	MTC, various slow-growing NTM	Bacteriostatic
	Pyrazinamide	MTC, with the exception of *M. bovis*	Bactericide (active and latent growth)
	Cycloserine	MTC (use restricted to MDR), variable in NTM	Bacteriostatic
	Delamanid	Use restricted to MDR	Bacteriostatic
Inhibition of protein synthesis	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
	Macrolides (clarithromycin, azithromycin)	Very effective in MAC. Active in NTM, except for *M. fortuitum* and *M. abscessus*. Of little use in MTC	Bacteriostatics
	Tetracyclines and tigecycline	Variable in NTM. Tigecycline more effective	Bacteriostatics
Inhibits folic acid synthesis	Dapsone	*M. leprae*	
	PAS (para-aminosalicylic acid)	MTC (use in MDR)	Bacteriostatic
	Co-trimoxazole	Variable in NTM	Bacteriostatic
Inhibits DNA synthesis	Fluoroquinolones (ofloxacin, levofloxacin, moxifloxacin)	MTC, NTM (MAC group 50%, rest variable)	Bactericides in active growth
Interferes in Fe metabolism	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*.