

Radiometric Susceptibility Testing of *Mycobacterium xenopi*

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Summary

Mycobacterium xenopi is an opportunistic pathogen, frequently isolated in various areas of Europe from pulmonary specimens, which may also cause infections in AIDS patients. We used the Bactec radiometric system (Becton Dickinson, USA) with a procedure expressly adapted to the particular growth characteristics of *M. xenopi* to determine the susceptibility patterns of 40 clinical isolates to six antimicrobial drugs. The majority of the strains were resistant to ethambutol and susceptible to amikacin, ciprofloxacin, rifampin, rifabutin and streptomycin.

Key words: *Mycobacterium xenopi*, susceptibility testing, radiometric method, minimum inhibitory concentration.

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INTRODUCTION

Mycobacterium xenopi is a slowly-growing, pigmented, acid-fast bacillus whose prevalence varies geographically: while, in fact, in the USA¹ the species can be considered somewhat rare, in several areas of Europe this microorganism is frequently isolated²⁻⁵. The well documented potential pathogenicity of *M. xenopi*⁶⁻⁹ is confirmed also by the reports of disseminated infections due to this species in AIDS patients¹⁰⁻¹².

No standardized technique for susceptibility testing has been developed so far for *M. xenopi*, as has been for all other species of mycobacteria other than tuberculosis. We report here the result of tests performed on 40 *M. xenopi* isolates using a method in radiometric liquid medium (Bactec, Becton Dickinson, Towson, USA) which follows the guidelines proposed by Heifets et al. for susceptibility testing of *Mycobacterium avium* complex (MAC)¹³ adapted to the peculiar growth characteristic of *M. xenopi*. The drugs tested include classical anti-tubercular substances like ethambutol, rifampin and streptomycin, new antimicrobials like rifabutin, and other substances recently used against mycobacteria, such as ciprofloxacin and amikacin.

MATERIALS AND METHODS

All the strains included in the study were isolated from clinical specimens: 26 from the sputum of patients with a respiratory pathology from which no other mycobacterium was isolated and 13 from AIDS patients (five blood cultures, six bronchial washings and two sputa).

From each patient no more than one isolate was tested.

Ethambutol, rifampin and streptomycin were prepared by rehydrating lyophilized substances (Bactec S.I.R.E., Becton Dickinson), amikacin was supplied by Pierrel (Capua, Italy), ciprofloxacin by Bayer (Milan, Italy) and rifabutin by Farmitalia Carlo Erba (Milan, Italy). Of every drug three different concentrations were tested (Table 1); stock solutions in distilled water (rifabutin was dissolved in methanol before the preparation of stock solution) were prepared with a concentration 40 times the final test concentration to be used so that the addition of 100 µl of each such solution to a vial of radiometric broth Bactec 12B containing 4 ml of medium, gave the required dilution.

Small amounts of growth were collected with a swab and suspended into Diluting Fluid (Becton Dickinson) to a McFarland standard no. 1. An aliquot of 100 µl of each suspension was inoculated into a "pre-inoculum" radiometric vial that was incubated at 42°C and read daily by Bactec 460 TB instrument until a growth index (GI) ≥ 999 was reached, which usually occurred within two days. The inoculation of vials containing drug as well as of drug-free control was made with 100 µl of the pre-inoculum broth whose GI had just exceeded 999. A second control vial was inoculated with a 1/100 dilution in Diluting Fluid of the pre-inoculum culture. All radiometric broths were incubated at 42°C.

For the radiometric evaluation of drug susceptibility all vials were read daily, approximately at the same hour, until the reading of the GI of the diluted control was over 20 for three consecutive days. The MIC, in this case the lowest concentration of a drug that inhibited more than 99% of the mycobacterial population, was taken as the lowest concentration in which, from the third day on, the daily GI remained lower than that of the diluted control and lower than 50, and the daily GI increment, from the third day on, was lower than the corresponding increment of the diluted control.

The following intra-assay control requirements were defined: i. the GI of the undiluted control must neither reach 999 before the third day, nor later than the tenth day (the day of inoculation was considered day 1), ii. the GI in the diluted control must be greater than 20

before the ninth day and remain such for three consecutive readings. When one or both of such rules were not fulfilled the test was repeated.

RESULTS AND DISCUSSION

The susceptibility of 40 strains of *M. xenophi* was quite homogeneous; the MICs of all the isolates were in fact high for ethambutol and low for all other drugs (Table 1).

The requirements of intra-assay control were fulfilled in 36 cases, while in the remaining four the test was repeated because in the first attempt an under-inoculation error was detected.

TABLE 1 - Per cent susceptibility of 40 *Mycobacterium xenophi* strains to various concentrations of six drugs.

Drug	Concentrations (µg/ml) ^a				
	0.5	1	2	4	8
Amikacin	nd	nd	100	100	100
Ciprofloxacin	nd	82.5	97.5	100	nd
Ethambutol	nd	nd	0	7.5	22.5
Rifabutin	97.5	nd	100	nd	100
Rifampin	85	nd	100	nd	100
Streptomycin	nd	nd	92.5	100	100

^a nd = concentration not tested

No difference in the pattern of susceptibility was detected between the isolates from AIDS and HIV-negative patients.

Even if *M. xenophi* is not uniformly present worldwide its prevalence in endemic areas is high. Moreover the reported isolation of this organism from AIDS patients deserves attention¹⁰⁻¹².

Available data concerning the antimicrobial susceptibility pattern of *M. xenophi* show almost absolute susceptibility to streptomycin and variable results for rifampin and ethambutol^{6,8,14}. They are, however, not easily interpretable as tests were performed with different antimicrobials and using techniques, on solid media, developed for *Mycobacterium tuberculosis*¹⁴. As stressed by Heifets¹⁵ the tests performed on liquid medium are the only ones that furnish accurate and precise results; moreover, only the

radiometric system makes it possible to achieve results in a short time.

The attempt to standardize radiometric susceptibility testing of MAC gave accurate and reproducible results in five reference centers in the USA and Canada¹⁶. We found it satisfactory and more affordable than other proposed techniques and we adopted it for the susceptibility testing of MAC (the results achieved on 52 isolates of *M. avium* and nine of *M. intracellulare* are reported in Table 2).

The changes in the original method were imposed by the peculiar growth characteristics of *M. xenopi*, which grows better at 42°C and with a slower rate in comparison with MAC, thus requiring a higher incubation temperature, a heavier inoculum and longer incubation. As the heavy inoculum adopted can induce, even in the presence of an effective antimicrobial, moderate growth that occasionally exceeds 50 during the first two days of incubation but that subsequently ceases, a reading over 50 within 48 h has not been considered as due to resistance.

TABLE 2 - Per cent susceptibility of 61 MAC strains (52 *Mycobacterium avium* and 9 *M. intracellulare*) to various concentrations of six drugs.

Drug	Concentrations (µg/ml) ^a				
	0.5	1	2	4	8
Amikacin	nd	nd	31	66	97
Ciprofloxacin	nd	5	6	16	nd
Ethambutol	nd	nd	20	82	95
Rifabutin	59	nd	89	nd	98
Rifampin	11	nd	26	nd	56
Streptomycin	nd	nd	34	67	93

^a nd = concentration not tested

The ranges of drug dilutions we adopted are those proposed by Heifets et al¹³, which correspond, at the lower limit, to the MICs found for wild *M. tuberculosis* strains, and at the upper limit, to the maximum drug concentrations achievable in blood or tissues. For *M. xenopi* such ranges were not suitable for the detection of exact MICs as higher concentrations for ethambutol and lower ones for all other drugs would be needed. They represent, however, useful breakpoints for the clinician as

they allow susceptibilities (inhibition by the lowest concentration) from resistance (tolerance of the highest) to be easily recognized.

No speculation is possible about correlations between *in vitro* and *in vivo* results; data from the literature report that the response to the most frequently administered treatment, a combination of isoniazid, ethambutol and rifampin, was variable^{6,17}.

The ineffectiveness of ethambutol alone against *M. xenopi* cannot, in our opinion, induce us to exclude this drug from therapeutic regimens. The ability of ethambutol, demonstrated for other mycobacterial species^{18,19}, to potentiate the efficacy of associated drugs by interfering with the synthesis of cell-wall lipids should be investigated.

Even if, from our data, the susceptibility pattern of *M. xenopi* was rather homogeneous and consequently easily foreseeable, the availability of an affordable method to test the susceptibility of this organism can be of interest, particularly in cases in which the inability of a therapeutic regimen to clear a patient from the infection leads to the suspicion that resistance has developed.

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