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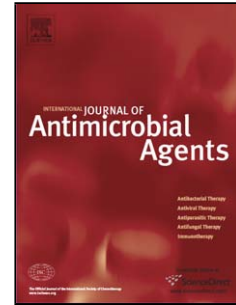
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In vitro drug susceptibility of 2275 clinical non-tuberculous mycobacteria isolates of 49 species in The Netherlands

Jakko van Ingen ^{a,b,*}, Tridia van der Laan ^b, Richard Dekhuijzen ^a, Martin Boeree ^a, Dick van Soolingen ^b

^a *Department of Pulmonary Diseases, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands*

^b *National Mycobacteria Reference Laboratory, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*

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* Corresponding author. Present address: RIVM; LIS (pb22), National Mycobacteria Reference Laboratory, Antonie van Leeuwenhoeklaan 9, P.O. Box 1, 3720 BA Bilthoven, The Netherlands. Tel.: +31 30 274 2886; fax: +31 30 274 4418.

E-mail address: jakko.van.ingen@rivm.nl (J. van Ingen).

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ABSTRACT

In this study, 2275 clinical isolates of 49 species of non-tuberculous mycobacteria isolated in The Netherlands were subjected to standardised drug susceptibility testing using the Middlebrook 7H10 agar dilution method. Clarithromycin and rifabutin were most active, with 87% and 83% of all isolates, respectively, being susceptible. Susceptibility to ciprofloxacin (44%) and amikacin (32%) was limited and was mostly restricted to *Mycobacterium kansasii*, *Mycobacterium xenopi*, *Mycobacterium fortuitum* and phylogenetically related species. Susceptibility to isoniazid (0.5%), rifampicin (37%), ethambutol (35%) and streptomycin (33%) was rare; susceptibility to cycloserine, clofazimine and prothionamide was generally restricted to slow growers, although prothionamide also had activity against *M. fortuitum* and related species. Significant discrepancies between in vitro and in vivo activity exist. To improve the utility of drug susceptibility testing, the selection of drugs should be changed to more drugs with proven clinical efficacy correlating with in vitro susceptibility.

1. Introduction

There is an increasing number of clinical isolates of non-tuberculous mycobacteria (NTM) in many countries as well as a growing awareness of their ability to cause disease [1,2]. NTM are opportunistic pathogens that can occasionally cause severe disease, usually in patients with pre-existing pulmonary disease or systemic impairment of immunity [1].

Treatment of NTM disease is time consuming and often complicated. Macrolide-based multidrug regimens are currently advocated by the American Thoracic Society (ATS) [1]. In a recent trial of the British Thoracic Society (BTS), no beneficial effect of macrolides on treatment of patients with pulmonary *Mycobacterium avium* complex (MAC), *Mycobacterium malmoense* and *Mycobacterium xenopi* disease was found [3]. Few clinical trials have been conducted, especially for disease due to less prevalent NTM species; current guidelines are mainly based on case reports and clinical experience.

Although there is a lack of correlation between in vitro drug susceptibility testing (DST) results and in vivo treatment outcome, DST is valuable, especially in patients with no response to first-line treatment or with a relapse of prior NTM disease [1]. For many of the infrequently isolated NTM species, no DST results have been published and there is little basis to predict potentially successful treatment regimens.

A variety of DST methods have been applied to NTM. The Clinical and Laboratory Standards Institute (CLSI) currently recommends broth-based

methods for MAC and related slow-growing NTM, with the broth microdilution technique also considered suitable for rapid growers [4]. Application of these techniques has highlighted the therapeutic potential of the macrolides, linezolid and tigecycline for disease due both to slow- and rapid-growing NTM [1,4].

In The Netherlands, a Middlebrook 7H10 agar dilution method has been used for over a decade with favourable results for *Mycobacterium tuberculosis* complex isolates [5]. This study reports the DST results on a wide variety of NTM isolated from clinical samples in The Netherlands and discusses the implications of these findings for NTM treatment regimens.

2. Materials and methods

DST results of all clinical NTM isolates subjected to laboratory diagnosis at the Dutch National Institute for Public Health and the Environment (RIVM), the national mycobacteria reference laboratory, were collected in the period between January 2000 and January 2007.

Identification to species level was performed by ruling out membership of the *M. tuberculosis* complex using the GenoType MTBC line-probe assay (Hain Lifescience, Nehren, Germany), followed by application of the INNO-LiPA MYCOBACTERIA v2 reverse line-blot (Innogenetics NV, Ghent, Belgium) for the more common NTM species. If identification to species level was not possible by these methods, 16S rDNA sequencing (151-bp hypervariable region A) was performed. Prior to 2004, 16S sequencing was performed for all

isolates after ruling out the *M. tuberculosis* complex, MAC and *M. avium* by the respective AccuProbe kits (GenProbe, San Diego, CA).

DST was performed using the 25-well agar dilution method, as recently published [5]. In short, dilutions of antimycobacterial drugs were mixed in liquefied 7H10 agar and filled out in 25-well plates. Following inoculation and incubation, bacterial growth at different concentrations of the antimycobacterial drugs was compared with that on agar in a well without the drug and inoculated with 1/100 of the inoculum. The minimum inhibitory concentration (MIC) is the lowest concentration of antimycobacterial drug to inhibit >99% of growth of the mycobacterial inoculum. Dilutions of antimycobacterial drugs in 7H10 agar were prepared with the following concentrations: 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 mg/L isoniazid (Sigma Chemical Co., St Louis, MO); 0.1, 0.2, 0.5, 1, 2 and 5 mg/L rifampicin (Sigma); 1, 2, 5, 10 and 20 mg/L streptomycin (Sigma); 1, 2, 5, 10 and 20 mg/L ethambutol (Sigma); 1, 2, 5, 10 and 20 mg/L amikacin (ICN Biomedicals Inc., Aurora, OH); 1, 2, 4, 8 and 16 mg/L ciprofloxacin (Bayer, Mijdrecht, The Netherlands); 2, 4, 8, 16 and 32 mg/L clarithromycin (Abbott, Chicago, IL); 2, 5, 10, 20 and 50 mg/L cycloserine (Sigma); 1, 2, 5, 10 and 20 mg/L prothionamide (Sanavita, Werne, Germany); 0.5, 1, 2, 5 and 10 mg/L clofazimine (Novartis Pharma, Breda, The Netherlands); and 0.2, 0.5, 1, 2 and 5 mg/L rifabutin (Pharmacia, Capelle a/d IJssel, The Netherlands).

The breakpoint concentrations are 1 mg/L for isoniazid and rifampicin, 5 mg/L for ethambutol, streptomycin, prothionamide and amikacin, 2 mg/L for

ciprofloxacin, rifabutin and clofazimine, 16 mg/L for clarithromycin and 50 mg/L for cycloserine [4]. Growth at the breakpoint concentration is reported as susceptible, and growth at higher concentrations of the drug is considered resistance. Isolates with MICs of 0.2 mg/L or 0.5 mg/L for isoniazid or of 5 mg/L or 10 mg/L for ethambutol and streptomycin are reported as 'intermediate resistant'.

For each series of isolates inoculated on DST plates, one *Mycobacterium gordonae* strain, one *M. avium* strain and three *M. tuberculosis* control strains that have been extensively characterised by our own laboratory were included as internal controls [5]. As a second-line quality control, strains were exchanged in a blind fashion with a peripheral laboratory and re-tested to check reproducibility.

3. Results

DST was performed on 2275 isolates of 49 different NTM species or complexes isolated from 2072 patients. The results obtained for published species of which five or more isolates were tested (2240 isolates of 29 species or complexes) are detailed qualitatively in Table 1. For this analysis, strains exhibiting intermediate susceptibility to isoniazid and ethambutol were considered resistant. The median MICs measured for all isolates are recorded in Table 2. The results obtained in the less frequent ($n < 5$) species are recorded in Table 3.

Isolates of most species were found to be susceptible to clarithromycin, most notably among the slow-growing NTM. Resistance to clarithromycin was only noted in the majority of *Mycobacterium simiae*, *Mycobacterium mageritense* and *M. fortuitum* isolates (Tables 1 and 2).

Clarithromycin susceptibility varied among MAC and *M. fortuitum* complex strains. Most *M. avium* strains had MICs around the breakpoint (16 mg/L) (Table 2), with a separate grouping (20%) exhibiting true clarithromycin resistance (MIC > 32 mg/L). For separate *Mycobacterium intracellulare* sequevars and MAC-X (MAC unidentified by the INNO-LiPA MYCOBACTERIA v2 reverse line-blot), median MICs were lower (Table 2). Within the *M. fortuitum* complex, *M. fortuitum* and *Mycobacterium peregrinum* could be separated, as *M. fortuitum* is resistant to clarithromycin and rifabutin whereas *M. peregrinum* was found to be susceptible (Table 1).

Resistance to the fluoroquinolone ciprofloxacin was more frequent and was noted in the majority of MAC, *Mycobacterium chelonae* and *Mycobacterium abscessus*, the phylogenetically related *M. malmoeense* and *Mycobacterium bohemicum* as well as in *M. simiae*, *Mycobacterium terrae* and *Mycobacterium celatum* isolates (Table 1).

Ciprofloxacin susceptibility was variable among *M. simiae*, *M. terrae*, *M. malmoeense* and *Mycobacterium mucogenicum* isolates. The MICs were either around the breakpoint concentration (*M. terrae*, *M. malmoeense*) (Table 2) or

there were separate strains with low and high MICs (*M. simiae*, *M. mucogenicum*).

In vitro susceptibility to first-line antituberculosis drugs, especially isoniazid and rifampicin, was found to be rare among NTM. However, susceptibility to rifabutin was frequent (Table 1). The phylogenetically related *Mycobacterium kansasii*, *Mycobacterium szulgai* and *Mycobacterium marinum* are susceptible to most classes of antimycobacterial drugs, as are the phylogenetically more distant *M. xenopi* and *M. goodnae*.

The *M. marinum*, *M. kansasii*, *M. szulgai* group and *M. xenopi*, *Mycobacterium noviomagense* and *M. celatum* were susceptible to amikacin. Susceptibility to the second-line antituberculosis drugs (clofazimine, cycloserine and prothionamide) was common, especially among slow-growing NTM. Slow- and rapid-growing NTM are divided by cycloserine susceptibility, with in vitro resistance restricted to the rapid growers, except for *Mycobacterium holsaticum*.

Serial isolates were available for 237 patients ($n = 587$; 2.5 per patient). For serial isolates, two-fold changes in MICs for any drug were common. Four-fold changes in MICs for any drug occurred in 5% of patients. This percentage was consistent among the different drugs tested.

Acquired resistance, defined as a resistant follow-up isolate after a susceptible primary isolate, was noted in 28 patients; acquired macrolide

resistance was most frequent (22 patients; 11 *M. avium*, 3 *M. intracellulare*, 2 MAC, 3 *M. abscessus*, 1 *M. chelonae*, 1 *M. kansasii* and 1 *M. malmoense*), followed by acquired rifampicin resistance ($n = 5$; 4 *M. malmoense* and 1 *M. szulgai*) and acquired ciprofloxacin resistance ($n = 3$; 1 *M. malmoense*, 1 *M. kansasii* and 1 *M. szulgai*).

4. Discussion

Analysing the results of 7 years of DST for NTM using the 25-well agar dilution method, it is evident that the results yield important clues for optimisation of NTM species-specific therapy.

The debate on the role of DST in the management of NTM disease is ongoing, mainly because of the observed discrepancies between in vitro susceptibility and in vivo response to treatment [1,3,6,7]. These discrepancies need further study, especially for the newer antimicrobial drugs and the less frequently isolated NTM species. Baseline testing for specific drugs such as rifampicin for *M. kansasii* and clarithromycin for MAC isolates helps to understand treatment failure or relapse and thereafter to select second-line treatment regimens [1,4].

Our in vitro results confirm the potential efficacy of the macrolides in the treatment of NTM disease. Only *M. fortuitum*, *M. mageritense* and *M. simiae* isolates are usually macrolide-resistant. Clinical trials of macrolide-based treatment for MAC disease in the USA have demonstrated superior results [6] compared with previous European trials using exclusively rifampicin and

ethambutol only [7]. However, a recent comparative trial of 2 years of rifampicin, ethambutol and clarithromycin or ciprofloxacin for pulmonary MAC, *M. malmoense* and *M. xenopi* disease was unable to demonstrate superiority of either triple-drug regimen drug over the rifampicin and ethambutol only regimen [3,6]. In this trial, in vitro susceptibility to ciprofloxacin or clarithromycin was not assessed [3]. We found frequent ciprofloxacin resistance among MAC (92–99%) and *M. malmoense* (74%) (Table 1) strains. Whilst casting doubt on the efficacy of ciprofloxacin, our results offer no explanation for the limited results in patients receiving a macrolide-based regimen in the BTS study [3]. This illustrates the necessity to acquire more clinical data that establish the correlation between in vitro and in vivo activity. There could be a role for macrolides in the case of first-line treatment failure or relapse with susceptible strains.

Although clarithromycin resistance in primary isolates was rare, 9% (22/237) of our patients with serial isolates had clarithromycin-resistant secondary isolates. In vitro resistance to macrolides has been related to poor in vivo treatment outcome [1,6].

For the macrolide-resistant species in our study, in vitro susceptibility to ciprofloxacin indicates a possible role for the fluoroquinolones. Most *M. simiae* isolates, however, are also resistant to ciprofloxacin (Table 1). Treatment results in *M. simiae* disease are, probably as a result, poor [8].

The common in vitro resistance to ciprofloxacin (Table 1) may explain its limited in vivo activity in the latest BTS trial [3]. Moxifloxacin is more active in vitro than ciprofloxacin [1] and may be active against isolates that have median MICs just above the ciprofloxacin breakpoint (*M. malmoense*, *M. chelonae*). Combination therapy of moxifloxacin and clarithromycin may not be advisable as fluoroquinolones were recently demonstrated to attenuate clarithromycin activity in a murine model of MAC disease [9]. How this translates to human disease remains to be determined.

The widespread amikacin resistance is contrary to results available in the literature, at least for *M. avium* and *M. abscessus* [1,4]. The discrepancies, which may result from the test methods or the applied breakpoint concentrations, are awkward since better sputum conversion rates have been recorded in patients receiving an aminoglycoside as adjunctive therapy for MAC disease [1,6]. Hence, reporting false amikacin resistance may negatively affect therapy choice and outcome.

In vitro susceptibility to cycloserine, prothionamide and clofazimine was common, especially among slow-growing NTM. However, the efficacy of these drugs in NTM treatment has not been sufficiently proven and they are limited by their toxicity [1,10,11].

The mechanisms by which resistance is mediated in NTM are not clear for all drugs. Macrolide resistance in MAC and rifampicin resistance in *M. kansasii* bacteria is mediated by mutations in the 23S rRNA and *rpoB* genes,

respectively [1,4,12]. For the rapid growers *M. fortuitum*, *Mycobacterium smegmatis* and *M. abscessus*, activation of an erythromycin methylase (*erm*) gene confers inducible macrolide resistance [1,13]. The high levels of intrinsic drug resistance may also result from the existence of multidrug efflux pumps [14].

Similar DST results may hint at a phylogenetic relationship between various species, for example *M. kansasii*, *M. szulgai* and *M. marinum* (Tables 1 and 2). The phylogenetic relationship of these species was also observed in a multigene taxonomical model [15].

The currently available technique and the range of drugs tested offer too little therapy guidance for clinicians and require improvement. Our results warrant a reconsideration of the drugs in the test panel and illustrate the necessity to acquire more clinical data that establish the correlation between in vitro and in vivo information. There should be separate test panels for slow- and rapid-growing NTM, and new promising antimycobacterial drugs, such as moxifloxacin, linezolid, the carbapenems and tigecycline, should be included in test panels and their breakpoints established. Standardised control strains should be used to allow interlaboratory comparisons. Alternatively, application of broth-based methods recommended by the CLSI may decrease the turnaround time and improve international standardisation.

In summary, the 25-well agar dilution method is an inexpensive and reliable method for DST of NTM. Results for the macrolides and fluoroquinolones

appear useful for therapy guidance. DST can assist in selecting second-line treatment regimens in cases of treatment failure or relapse. Most NTM species show in vitro susceptibility to macrolides. The conflicting results of the clinical trials render their role in clinical practice unclear. This illustrates the necessity to acquire more clinical data that establish the correlation between in vitro and in vivo activity. One could defend a recommendation to use macrolides in the case of treatment failure or relapse with susceptible strains. Acquired resistance, mainly to the macrolides, is a significant issue. A reconsideration of the test platform and choice of drugs is needed to improve its utility, especially for rapid-growing mycobacteria. The utility of first- and second-line antituberculosis drug testing is very limited and this should be substituted for newer drugs with proven clinical efficacy correlating with in vitro susceptibility. The DST results have additional taxonomical value.

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Table 1Percentage of non-tuberculous mycobacteria strains ($n \geq 5$) resistant in vitro to drugs in the test panel, per species ^a

Species	n^b	INH	RIF	ETH	STR	RIB	AMI	CIP	CLA	CYC	CLO	PRO
<i>M. avium</i>	688	100	99	49	100	14	100	95	20	5	7	4
MAC	118	99	96	75	99	14	98	92	22	2	8	3
MAC-X	29	93	79	97	86	4	97	90	4	3	0	24
<i>M. intracellulare</i>	201	99	95	86	99	6	98	99	4	15	2	9
<i>M. paraffinicum</i>	8	100	88	50	100	0	100	100	0	0	0	25
<i>M. scrofulaceum</i>	8	100	100	75	100	0	100	100	0	0	0	0
<i>M. haemophilum</i>	49	100	96	100	65	4	71	12	6	22	92	39
<i>M. bohemicum</i>	8	100	0	75	88	0	100	88	0	0	0	0
<i>M. malmoense</i>	90	100	32	57	80	1	79	74	3	4	1	2
<i>M. interjectum</i>	12	100	42	83	67	0	100	17	0	0	0	0
<i>M. lentiflavum</i>	11	100	100	64	82	0	100	9	9	9	9	9
<i>M. simiae</i>	29	100	97	97	100	97	100	62	75	0	10	34
<i>M. marinum</i>	61	100	3	5	7	3	5	12	3	3	0	3
<i>M. kansasii</i>	262	100	2	8	15	0	54	15	1	1	0	0
<i>M. szulgai</i>	23	100	4	9	9	0	13	26	0	13	0	0
<i>M. terrae</i>	11	100	82	9	82	0	91	82	9	0	64	27

<i>M. xenopi</i>	50	94	18	56	0	0	6	0	0	2	0	0
<i>M. noviomagense</i>	10	100	90	90	0	0	0	0	0	0	0	0
<i>M. celatum</i>	15	53	100	53	7	93	40	80	7	13	7	0
<i>M. gordonae</i>	278	81	2	4	15	0	15	1	0	1	0	0
<i>M. abscessus</i>	82	100	100	99	100	93	95	93	38	99	90	93
<i>M. chelonae</i>	54	98	98	100	96	94	96	59	19	92	80	67
<i>M. mageritense</i>	6	100	100	33	83	100	83	0	83	100	83	100
<i>M. fortuitum</i>	46	96	98	67	96	83	44	4	83	96	46	33
<i>M. peregrinum</i>	23	100	96	52	70	22	4	0	5	91	30	35
MFC	42	100	95	43	71	62	10	5	58	93	60	31
<i>M. mucogenicum</i>	15	93	73	40	73	33	20	33	7	73	53	60
<i>M. alvei</i>	5	100	100	60	60	40	0	0	40	80	20	60
<i>M. holsaticum</i>	6	100	100	17	0	0	0	0	0	0	17	0

INH, isoniazid; RIF, rifampicin; ETH, ethambutol; STR, streptomycin; RIB, rifabutin; AMI, amikacin; CIP, ciprofloxacin; CLA, clarithromycin; CYC, cycloserine; CLO, clofazimine; PRO, prothionamide; MAC, *Mycobacterium avium* complex; MAC-X, MAC unidentified by the INNO-LiPA MYCOBACTERIA v2 reverse line-blot; MFC, *M. fortuitum* complex.

^a All data are the percentage of strains that are found to be resistant to the drug tested; bold face indicates that the majority are susceptible.

^b Number of strains tested.

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Table 2

Median minimum inhibitory concentrations (mg/L) for non-tuberculous mycobacteria ($n \geq 5$) of all drugs included in the panel, per species

Species	n^a	INH	RIF	ETH	STR	RIB	AMI	CIP	CLA	CYC	CLO	PRO
<i>M. avium</i>	688	5	>5	10	>20	2	>20	>16	16	20	1	≤ 1
MAC	118	5	>5	20	>20	2	>20	>16	16	50	1	2
MAC-X	29	5	>5	10	>20	1	>20	>16	4	20	≤ 0.5	≤ 1
<i>M. intracellulare</i>	201	5	>5	10	>20	1	>20	>16	8	50	≤ 0.5	2
<i>M. paraffinicum</i>	8	5	>5	20	10	1	>20	4	4	20	≤ 0.5	≤ 1
<i>M. scrofulaceum</i>	8	10	5	20	20	1	>20	>16	4	20	1	≤ 1
<i>M. haemophilum</i>	49	>20	≤ 0.1	>20	10	≤ 0.2	>20	≤ 1	≤ 2	50	≤ 0.5	2
<i>M. bohemicum</i>	8	>20	0.5	20	20	≤ 0.2	>20	16	≤ 2	≤ 2	≤ 0.5	2
<i>M. malmoense</i>	90	5	1	20	>20	≤ 0.2	>20	4	≤ 2	50	≤ 0.5	≤ 1
<i>M. interjectum</i>	12	5	0.5	10	10	≤ 0.2	10	≤ 1	≤ 2	10	≤ 0.5	≤ 1
<i>M. lentiflavum</i>	11	1	>5	20	10	1	20	≤ 1	16	≤ 5	1	≤ 1
<i>M. simiae</i>	29	10	>5	20	>20	>5	>20	2	>32	50	1	5
<i>M. marinum</i>	61	10	0.2	2	2	≤ 0.2	2	≤ 1	≤ 2	10	≤ 0.5	≤ 1
<i>M. kansasii</i>	262	1	0.2	5	5	≤ 0.2	10	2	≤ 2	10	≤ 0.5	≤ 1

<i>M. szulgai</i>	23	1	0.5	5	5	0.5	5	2	≤2	50	≤0.5	≤1
<i>M. terrae</i>	11	>20	>5	5	20	1	>20	8	≤2	50	5	5
<i>M. xenopi</i>	50	0.5	0.5	20	≤1	≤0.2	5	≤1	≤2	50	≤0.5	≤1
<i>M. noviomagense</i>	10	0.5	2	10	≤1	≤0.2	2	≤1	≤2	50	≤0.5	≤1
<i>M. celatum</i>	15	2	>5	10	5	>5	5	8	≤2	50	1	≤1
<i>M. gordonae</i>	278	2	0.2	2	5	≤0.2	5	≤1	≤2	20	≤0.5	≤1
<i>M. abscessus</i>	82	>20	>5	>20	>20	>5	>20	8	≤2	>50	5	>20
<i>M. chelonae</i>	54	>20	>5	>20	>20	>5	>20	4	≤2	>50	5	>20
<i>M. mageritense</i>	6	>20	>5	10	>20	5	>20	≤1	>32	>50	5	>20
<i>M. fortuitum</i>	46	5	>5	20	>20	>5	5	≤1	>32	>50	2	2
<i>M. peregrinum</i>	23	2	>5	20	20	2	≤1	≤1	≤2	>50	2	2
MFC	42	>20	>5	10	>20	>5	5	≤1	>32	>50	5	2
<i>M. mucogenicum</i>	15	>20	>5	2	20	0.5	5	≤1	≤2	>50	5	>20
<i>M. alvei</i>	5	>20	>5	10	20	2	2	≤1	≤2	>50	2	>20
<i>M. holsaticum</i>	6	1	>5	≤1	≤1	1	≤1	≤1	≤2	20	1	2

INH, isoniazid; RIF, rifampicin; ETH, ethambutol; STR, streptomycin; RIB, rifabutin; AMI, amikacin; CIP, ciprofloxacin; CLA, clarithromycin; CYC, cycloserine; CLO, clofazimine; PRO, prothionamide; MAC, *Mycobacterium avium* complex; MAC-X, (MAC unidentified by the INNO-LiPA MYCOBACTERIA v2 reverse line-blot; MFC, *M. fortuitum* complex.

^a Number of strains tested.

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Table 3Drug susceptibility testing results of seldom encountered non-tuberculous mycobacteria ($n < 5$)

Species	n^a	INH	RIF	ETH	STR	RIB	AMI	CIP	CLA	CYC	CLO	PRO
<i>M. conspicuum</i>	4	R	R	S	S	S	R	S	S	S	S	S
<i>M. palustre</i>	4	R	S	I	R	S	R	S	S	S	S	S
<i>M. gilvum</i>	2	R	S/R	S	S	S	S	S	S	S	S	S
<i>M. hiberniae</i>	2	R	R	S	S	S	S	S	S	S	S	S
<i>M. cosmeticum</i>	1	R	R	I	R	R	S	R	S	S	R	R
<i>M. florentinum</i>	1	R	R	I	R	S	R	R	S	S	R	S
<i>M. hassiacum</i>	1	I	S	S	S	S	S	S	S	S	S	S
<i>M. neoaurum</i>	1	R	S	R	S	S	S	S	S	R	S	R
<i>M. nonchromogenicum</i>	2	R	R	S	S	S	R	S	S	S	S/R	S
<i>M. novocastrense</i>	1	I	R	S	S	S	S	S	S	S	S	S
<i>M. obuense</i>	2	R	R	R	S	S	S	S	S	R	S	S/R
<i>M. phlei</i>	3	R	R	S	S	S	S	S	S	S	R	R
<i>M. porcinum</i>	1	R	R	R	R	R	S	S	R	R	R	R
<i>M. shimoidei</i>	1	I	R	S	S	S	S	S	S	S	S	S
<i>M. smegmatis</i>	1	R	R	S	S	R	S	S	R	R	S	R
<i>M. sphagni</i>	1	R	R	S	S	S	S	S	S	S	S	R

<i>M. vanbaalenii</i>	1	R	R	S	S	S	S	S	S	S	S	S
<i>M. wolinskyi</i>	2	R	R	S	S/R	S	R	S	R	R	S	R
<i>M. nebraskense</i>	1	R	S	S	R	S	R	S	S	S	S	R
<i>M. heidelbergense</i>	3	I	R	I	S	S	R	S	S	S	S	S

INH, isoniazid; RIF, rifampicin; ETH, ethambutol; STR, streptomycin; RIB, rifabutin; AMI, amikacin; CIP, ciprofloxacin; CLA, clarithromycin; CYC, cycloserine; CLO, clofazimine; PRO, prothionamide; R, resistant; S, susceptible; I, intermediate-susceptible.

^a Number of strains tested.