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Original Article

In vitro drug susceptibility of 40 international reference rapidly growing mycobacteria to 20 antimicrobial agents

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Abstract: Rapidly growing mycobacteria (RGM) are human pathogens that are relatively easily identified by acid-fast staining but are proving difficult to treat in the clinic. In this study, we performed susceptibility testing of 40 international reference RGM species against 20 antimicrobial agents using the cation-adjusted Mueller-Hinton (CAMH) broth microdilution based on the minimum inhibitory concentration (MIC) assay recommended by the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The results demonstrated that RGM organisms were resistant to the majority of first-line antituberculous agents but not to second-line fluoroquinolones or aminoglycosides. Three drugs (amikacin, tigecycline and linezolid) displayed potent antimycobacterial activity against all tested strains. Capreomycin, levofloxacin and moxifloxacin emerged as promising candidates for the treatment of RGM infections, and cefoxitin and meropenem were active against most strains. *Mycobacterium chelonae (M. chelonae)*, *M. abscessus*, *M. bolletii, M. fortuitum, M. boenickei, M. conceptionense, M. pseudoshottsii, M. septicum* and *M. setense* were the most resistant RGM species. These results provide significant insight into the treatment of RGM species and will assist optimization of clinical criteria.

Keywords: Drug susceptibility, reference strains, rapidly growing mycobacteria (RGM), antimicrobial, minimum inhibitory concentration (MIC)

Introduction

All members of the genus *Mycobacterium* with the exception of *Mycobacterium tuberculosis* and *M. leprae* are considered nontuberculous mycobacteria (NTM) [1]. More than 160 species of NTM are documented in the Genus Mycobacterium database (http://www.bacterio.cict.fr/m/mycobacterium.html; accessed on 22 August 2013). These species are ubiquitously distributed in the environment in fresh and salt water, soil and biofilms [2]. Among NTM, rapidly growing mycobacteria (RGM) have recently gained increasing attention because they are associated with specific diseases and are characterized by extensive resistance to antimicrobial drugs [1]. RGMs are diverse and include *Mycobacterium abscessus* [3], *M. chelonae*, *M. fortuitum* [1], *M. immunogenum*, and *M. smegmatis* [1] groups. These strains can be cultured rapidly in as little as seven days [4, 5]. To date, more than 50 species of RGM have been identified [6], many of which cause a broad spectrum of human such as pulmonary infections that resemble tuberculosis, infections in skin, soft tissue and the bloodstream, and osteomyelitis [1, 7, 8]. Among disease-causing RGM, *M. abscessus* is the most important respiratory pathogen and accounts for approximately 80% of RGM respiratory disease isolates [1].

Treatment of infections caused by RGM remains difficult [9] not least due to the difficulty of

Table 1. MIC (μg/ml) breakpoints of 20 selected antimicrobial agents

Drug	MIC breakpoint		
	Susceptibility	Moderate susceptibility	Resistance
Rifampicin	—	—	≥ 1
Isoniazid	—	—	≥ 1
Ethambutol	—	—	≥ 4
Streptomycin	—	—	≥ 5
Amikacin	≤ 16	32	≥ 64
Kanamycin	—	—	≥ 4
Capreomycin	—	—	≥ 2.5
Tobramycin	≤ 2	4	≥ 8
Ofloxacin	—	—	≥ 2
Ciprofloxacin	≤ 1	2	≥ 4
Levofloxacin	≤ 2	4	≥ 8
Moxifloxacin	≤ 1	2	≥ 4
Linezolid	≤ 8	16	≥ 32
Sulfamethoxazole	≤ 38	—	≥ 76
Minocycline	≤ 1	2-4	≥ 8
Clarithromycin	≤ 2	4	≥ 8
Doxycycline	≤ 1	2-4	≥ 8
Tigecycline	≤ 1	2-4	≥ 8
Cefoxitin	≤ 16	32-64	≥ 128
Meropenem	≤ 4	8-16	≥ 32

selecting the appropriate antimicrobial therapy [5]. In this study, we used the cation-adjusted Mueller-Hinton (CAMH) broth microdilution method to measure the minimum inhibitory concentration (MIC) of 20 antibiotic agents against 40 international reference RGM species *in vitro* to identify effective drugs for each species. The results may prove useful for clinical diagnosis and treatment of RGM, and contribute to international criteria for drug susceptibility patterns.

Materials and methods

Strains

40 international reference RGM strains were purchased from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung Von Mikroorganismen and Zellkulturen, DSMZ) and the American Type Culture Collection (ATCC). *Mycobacterium chelonae* and *M. pseudoshottsii* were cultured at 32°C and 25°C, respectively, and all other strains were incubated at 37°C.

Antimicrobial agents

20 antimicrobial agents including rifampicin (RFP), isoniazid (INH), ethambutol (EMB), streptomycin (SM), amikacin (AM), kanamycin (KN), capreomycin (CPM), tobramycin (TOB), ofloxacin (OF), ciprofloxacin (CIP), levofloxacin (LEV), moxifloxacin (MOX), linezolid (LN), clarithromycin (CLR), sulfamethoxazole (SMZ), cefoxitin (FOX), minocycline (MIN), doxycycline (DOX), tigecycline (TIG) and meropenem (MEM) were purchased from Sigma-Aldrich (St. Louis, USA). Alamar Blue was obtained from Bio-Rad Corporation (California, USA). All antimicrobial solutions were prepared the day before the experiment and stored at -70°C.

Drug susceptibility test

Strains were incubated on 7H10 agar and the drug susceptibility test was performed using the CAMH broth microdilution method in accordance with the standard operation procedures of the CLSI [10]. All experiments were repeated at least twice in 96-well microtitre plates. MICs of each antimicrobial agent for each strain are reported as the average of the two tests. Bacteria were adjusted using saline to a density of 0.5 McFarland standard units (1×10^7 colony forming units (CFU)/ml), and 50 μL of bacterial suspension were transferred to 10 ml of CAMH broth and vortexed thoroughly. Antimicrobial drugs were successively diluted two-fold in 100 μL CAMH broth and mixed with 100 μL of bacterial suspension to give the following final drug concentrations: rifampicin, isoniazid, ethambutol, streptomycin, tobramycin, sulfamethoxazole, cefoxitin, tigecycline and meropenem were 0.25-256 μg/mL; amikacin, kanamycin, capreomycin, ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin, clarithromycin, doxycycline and minocycline were 0.03-32 μg/mL; linezolid was 0.06-64 μg/mL. Two negative controls (drug free, CAMH + bacteria; bacteria free, CAMH only) were included to define the appropriate time to add the Alamar Blue (drug free) and to quantify the level of interference from the CAMH media (bacteria free). 96-well microtitre plates were sealed in individual plastic bags and incubated at the appropriate temperatures in controlled incubators. Plates were checked after 72 h and the indicator (20 μL of

Table 2. MICs (μg/mL) from antimicrobial susceptibility testing of 40 international reference rapidly growing mycobacteria

Sp. (international code)	INH	RFP	EMB	SM	AM	KN	CPM	TOB	OF	CIP	LEV	MOX	LN	SMZ	MIN	DOX	TIG	CLR	FOX	MEM
M. chelonae (ATCC35752)	> 256	> 256	128	32	4	16	16	2	32	4	16	4	8	64	> 32	> 32	1	0.06	128	> 256
M. abscessus (ATCC19977)	> 256	64	32	16	1	2	0.5	8	4	2	1	1	4	32	8	32	4	< 0.03	32	64
M. bolletii (DSM45149)	> 256	32	64	16	0.5	0.5	1	8	16	4	4	1	2	32	> 32	32	0.06	< 0.03	32	0.25
M. massiliense (DSM45103)	> 256	32	64	2	0.13	2	0.03	32	0.06	0.03	0.03	0.03	4	64	8	0.5	0.06	0.03	64	16
M. fortuitum (DSM44220)	64	128	256	32	0.25	4	< 0.03	16	0.13	< 0.03	< 0.03	< 0.03	8	2	8	0.03	0. 5	1	32	4
M. senegalense (ATCC35796)	> 256	8	64	4	0.5	0.25	0.13	4	4	4	2	0.13	1	16	0.25	0.06	0.03	0.13	4	2
M. boenickei (DSM44677)	32	128	4	8	0.5	4	0.06	4	0.5	0.25	0.25	0.06	8	4	> 32	16	0.03	16	16	0.5
M. goodii (DSM44492)	> 256	32	2	32	4	16	0.06	32	0.5	0.5	0.25	0.06	1	8	2	0.5	0.13	> 32	8	0.25
M. wolinskyi (DSM44493)	> 256	64	128	16	0.5	2	0.13	32	0.5	0.5	0.5	0.25	0.5	4	1	0.13	0.25	> 32	32	1
M. aichiense (ATCC27280)	> 256	< 0.25	0.5	< 0.25	0.06	0.03	< 0.03	0.5	< 0.03	< 0.03	< 0.03	< 0.03	0.5	2	0.25	< 0.03	0.03	< 0.03	0.5	0.25
M. aurum (ATCC23366)	> 256	4	1	< 0.25	0.25	0.06	< 0.03	4	0.06	0.03	< 0.03	< 0.03	0.5	4	0.25	0.03	0.13	0.06	16	16
M. chubuense (ATCC27278)	> 256	1	0.5	0.5	0.25	0.03	< 0.03	2	0.03	< 0.03	< 0.03	< 0.03	0.25	1	0.25	0.13	0.13	< 0.03	128	16
M. duvalii (ATCC43910)	> 256	0.5	0.25	0.5	0.25	0.5	< 0.03	2	0.06	< 0.03	0.06	< 0.03	0.5	8	0.5	0.03	0.06	0.5	8	2
M. flavescens (ATCC14474)	> 256	32	0.5	1	0.13	0.13	< 0.03	2	0.13	< 0.03	0.06	< 0.03	0.5	0.5	0.5	0.06	0.03	8	8	64
M. gilvum (ATCC43909)	> 256	0.25	0.25	1	0.25	0.25	0.03	1	0.13	< 0.03	0.03	0.03	0.25	4	0.25	0.03	0.06	0.03	4	2
M. neoaurum (ATCC25795)	> 256	1	8	0.5	0.5	0.06	< 0.03	0.25	0.06	0.06	0.03	< 0.03	0.5	0.5	0.03	0.06	0.03	1	8	0.5
M. obuense (ATCC27023)	> 256	2	64	< 0.25	0.13	0.13	< 0.03	1	< 0.03	< 0.03	< 0.03	< 0.03	0.5	32	0.25	< 0.03	0.06	< 0.03	< 0.25	0.5
M. parafortuitum (ATCC19686)	0.5	2	2	0.5	0.5	0.5	< 0.03	0.5	0.06	0.06	0.06	< 0.03	0.5	2	0.25	0.03	0.06	1	4	0.25
M. rhodesiae (ATCC27024)	8	< 0.25	< 0.25	< 0.25	0.13	< 0.03	< 0.03	0.25	0.06	< 0.03	0.03	< 0.03	0.25	1	0.25	0.06	0.03	< 0.03	< 0.25	1
M. tokaiense (ATCC27282)	4	< 0.25	0.5	0.5	0.25	0.25	< 0.03	0.5	0.06	0.03	0.03	< 0.03	0.5	8	0.25	0.13	0.03	0.13	128	0.25
M. porcinum (ATCC33776)	16	64	64	16	0.5	1	0.13	2	1	0.25	0.5	0.06	8	4	16	16	0.06	2	8	4
M. pulveris (ATCC35154)	> 256	64	4	2	0.5	0.13	0.03	2	> 32	0.5	1	0.13	2	256	0.25	0.06	0.03	2	256	1
M. austroafricanum (ATCC33464)	16	1	0.5	< 0.25	0.25	0.06	0.03	2	0.25	0.13	0.06	0.03	0.25	256	0.25	0.03	0.13	<0.03	8	0.5
M. chitae (ATCC19627)	> 256	8	< 0.25	0.5	0.13	0.03	< 0.03	0.5	0.03	< 0.03	< 0.03	< 0.03	0.5	0.5	2	0.03	0.06	4	4	4
M. aubagnense (DSM45150)	> 256	2	< 0.25	1	0.06	0.5	< 0.03	1	0.25	0.13	0.06	0.06	0.25	1	16	2	< 0.03	< 0.03	2	0.25
M. brisbanense (DSM44680)	> 256	128	> 256	16	0.13	2	0.13	8	0.5	0.25	0.25	0.06	0.5	4	16	4	0.13	0.13	64	4
M. brumae (DSM44177)	> 256	128	64	32	0.06	< 0.03	< 0.03	0.5	< 0.03	< 0.03	< 0.03	< 0.03	1	2	0.25	< 0.03	0.25	< 0.03	64	32
M. canariasense (DSM44828)	16	4	1	0.5	0.5	0.5	0.06	0.25	0.5	0.25	0.25	0.03	0.5	0.5	0.25	0.13	0.13	0.13	4	4
M. conceptionense (DSM45102)	> 256	128	256	32	0.5	1	0.06	4	0.5	0.25	0.25	0.06	16	128	0.5	0.13	< 0.03	0.13	128	0.25
M. confluentis (DSM44017)	> 256	8	32	4	0.13	0.03	< 0.03	0.25	0.03	< 0.03	< 0.03	< 0.03	2	1	1	0.06	0.25	2	4	2
M. fluoranthenivorans (DSM44556)	> 256	0.5	0.5	< 0.25	0.06	0.13	< 0.03	1	0.06	< 0.03	0.03	< 0.03	0.25	64	0.25	0.06	0.25	< 0.03	2	16
M. moriokaense (DSM44221)	> 256	8	16	8	2	0.25	0.25	8	> 32	0.5	0.5	0.03	2	64	0.25	0.03	0.03	0.5	16	2
M. mucogenicum (DSM44625)	0.5	32	16	8	1	2	0.5	4	2	1	1	0.25	2	16	0.25	0.06	< 0.03	0.06	16	0.25
M. poriferae (DSM44585)	> 256	128	128	16	4	1	0.13	8	1	2	2	0.13	1	4	0.25	0.25	0.03	< 0.03	32	2
M. pseudoshottsii (DSM45108)	> 256	256	128	32	2	0.5	1	4	8	2	4	1	8	4	0.25	32	< 0.03	0.03	> 256	128
M. psychrotolerans (DSM44697)	4	< 0.25	< 0.25	< 0.25	0.06	< 0.03	< 0.03	4	< 0.03	< 0.03	< 0.03	< 0.03	0.03	0.25	1	0.13	0.03	< 0.03	< 0.25	2
M. septicum (DSM44393)	256	16	4	16	0.13	0.5	< 0.03	1	0.25	0.06	0.13	0.06	1	1	32	8	1	1	128	16
M. setense (DSM45070)	> 256	64	16	8	0.5	1	< 0.03	4	0.25	0.06	0.13	0.03	4	> 256	> 32	> 32	0.13	4	16	0.25
M. vanbaalenii (DSM7251)	> 256	1	0.5	< 0.25	0.13	0.13	0.25	1	0.25	0.13	0.13	0.03	0.25	8	16	< 0.03	0.25	< 0.03	4	64
M. murale (DSM44340)	> 256	< 0.25	0.25	0.5	0.13	1	< 0.03	1	0.03	< 0.03	< 0.03	< 0.03	0.5	2	0.25	0.13	0.13	0.06	16	32
Standard Deviation	74.64	59.64	67.62	11.78	1.05	3.62	3.54	8.36	6.1	1.3	3	0.85	3.38	59.94	7.09	9.62	0.69	3.48	56.2	25.94

Note 1: INH: isoniazid, RFP: rifampicin, EMB: ethambutol, SM: streptomycin, AM: amikacin, KN: kanamycin, CPM: capreomycin, TOB: tobramycin, OF: ofloxacin, CIP: ciprofloxacin, LEV: levofloxacin, MOX: moxifloxacin, LN: linezolid, SMZ: sulfamethoxazole, MIN: minocycline, CLR: clarithromycin, DOX: doxycycline, TIG: tigecycline, FOX: cefoxitin, MEM: meropenem. Note 2: Numbers in bold type and underlined indicate sensitive strains; numbers in bold type only indicate moderately sensitive strains. Note 3: *M.* stands for *Mycobacterium*.

Table 3. Susceptibility of 40 international reference rapidly growing mycobacterial strains towards 20 selected antimicrobial agents

Sp. (international code)	INH	RFP	EMB	SM	AM	KN	CPM	TOB	OF	CIP	LEV	MOX	LN	SMZ	MIN	DOX	TIG	CLR	FOX	MEM	Susceptibility rate (%)
M. chelonae (ATCC35752)	+	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	-	-	+	+	25
M. abscessus (ATCC19977)	+	+	+	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	+	55
M. bolletii (DSM45149)	+	+	+	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	55
M. massiliense (DSM45103)	+	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	70
M. fortuitum (DSM44220)	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	70
M. senegalense (ATCC35796)	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	75
M. boenickei (DSM44677)	+	+	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	+	-	-	60
M. goodii (DSM44492)	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	70
M. wolinskyi (DSM44493)	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	70
M. aichiense (ATCC27280)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. aurum (ATCC23366)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90
M. chubuense (ATCC27278)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	85
M. duvalii (ATCC43910)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. flavescens (ATCC14474)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	80
M. gilvum (ATCC43909)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. neoaurum (ATCC25795)	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85
M. obuense (ATCC27023)	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85
M. parafortuitum (ATCC19686)	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. rhodesiae (ATCC27024)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. tokaiense (ATCC27282)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	90
M. porcinum (ATCC33776)	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	70
M. pulveris (ATCC35154)	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	70
M. austroafricanum (ATCC33464)	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	85
M. chitae (ATCC19627)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90
M. aubagnense (DSM45150)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	85
M. brisbanense (DSM44680)	+	+	+	+	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	70
M. brumae (DSM44177)	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	75
M. canariasense (DSM44828)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90
M. conceptionense (DSM45102)	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	70
M. confluentis (DSM44017)	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85
M. fluoranthenivorans (DSM44556)	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	90
M. moriokaense (DSM44221)	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	65
M. mucogenicum (DSM44625)	-	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	80
M. poriferae (DSM44585)	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	75
M. pseudoshottsii (DSM45108)	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	60
M. psychrotolerans (DSM44697)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	95
M. septicum (DSM44393)	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	65
M. setense (DSM45070)	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	65
M. vanbaalenii (DSM7251)	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	80
M. murale (DSM44340)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	90

Note 1: + = resistant; - = susceptible. Note 2: *M.* stands for *Mycobacterium.*

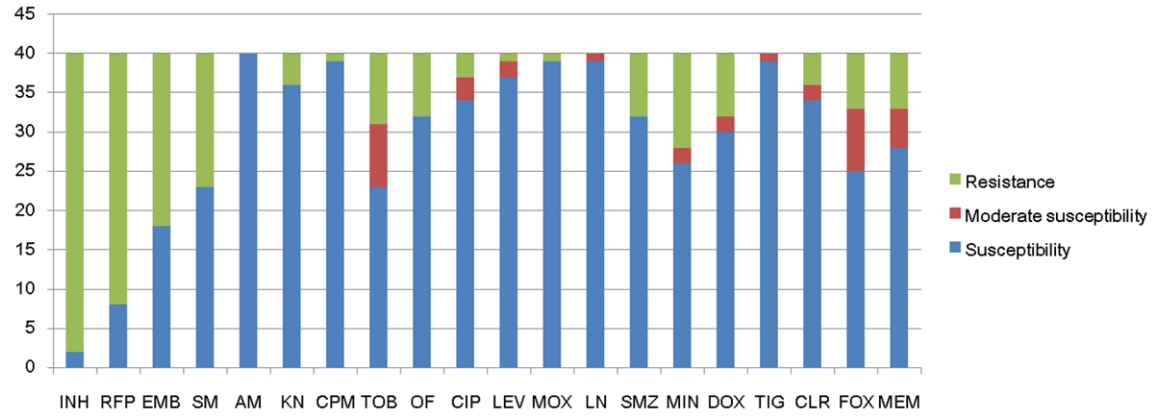

Figure 1. Susceptibility of 40 reference rapidly growing mycobacteria to 20 selected antimicrobial agents.

Alamar Blue and 50 μL of sterile 5% Tween-80) turned pink (provided the drug-free growth control was sufficient). MICs were usually measured on day 3 or 4 and tests were repeated if growth in the drug-free control was insufficient on day 5. MIC breakpoints of antibiotics indicating susceptibility, moderate susceptibility and resistance were interpreted according to the approved guidelines established by the CLSI [10] and the World Health Organization (WHO) [11] (**Table 1**).

Statistical analysis

Final MIC values for each antimicrobial agent are reported as means ± standard deviation as calculated using SPSS 17.0 software.

Results

MICs were determined for 40 international reference RGM strains against 20 selected antimicrobial agents (**Table 2**). Among the four first-line antituberculous agents, 38/40 strains (95%) were highly resistant to isoniazid, and 32/40 strains (80%) presented resistance to rifampicin. A higher percentage of strains were susceptible to ethambutol (22/40, 55%) and streptomycin (18/40, 45%). Fluoroquinolones, which include ofloxacin (32/40, 80%), ciprofloxacin (37/40, 92.50%), levofloxacin (39/40, 97.50%) and moxifloxacin (39/40, 97.50%) exhibited powerful *in vitro* activity against most RGM strains tested. However, *M. chelonae* was resistant to all four fluoroquinolone agents. Aminoglycosides including amikacin (40/40, 100%), kanamycin (36/40, 90%), capreomycin (39/40, 97.50%) and tobramycin (31/40, 77.50%) were also effective *in vitro*, and all strains demonstrated complete susceptibility to tigecycline (40/40, 100%) and linezolid (40/40, 100%).

Clarithromycin (36/40, 90%) showed excellent activity against all RGM organisms except *M. boenickei, M. goodie* and *M. wolinskyi*. Minocycline (29/40, 72.50%) and doxycycline (32/40, 80%) also displayed significant *in vitro* antimycobacterial activity but *M. chelonae*, *M. abscessus*, *M. boenickei*, *M. bolletii, M. septicum* and *M. setense* showed resistance. Cefoxitin (33/40, 82.50%) and meropenem (33/40, 82.50%) exhibited considerable activity against all RGM strains excluding *M. chelonae* and *M. pseudoshottsii*, while sulfamethoxazole (32/40, 80%) inhibited all strains apart from *M. chelonae, M. pulveris, M. austroafricanum, M. conceptionense* and *M. setense*.

Susceptibility rates were determined for the 20 selected antimicrobial agents against the 40 international reference RGMs (**Table 3**), and the most susceptible species were *M. aichiense*, *M. duvalii*, *M. gilvum*, *M. parafortuitum*, *M. rhodesiae* and *M. psychrotolerans*. The most resistant species was *M. chelonae*.

Antibacterial susceptibility distributions were determined for al 40 RGMs (**Figure 1**), and amikacin, linezolid and tigecycline scored 100% susceptibility, meaning all strains were susceptible to these agents. Capreomycin, levofloxacin and moxifloxacin were also among the highest scoring for susceptibility and are therefore good candidates for the treatment of RGM infections. *M. abscessus* and *M. bolletii* were

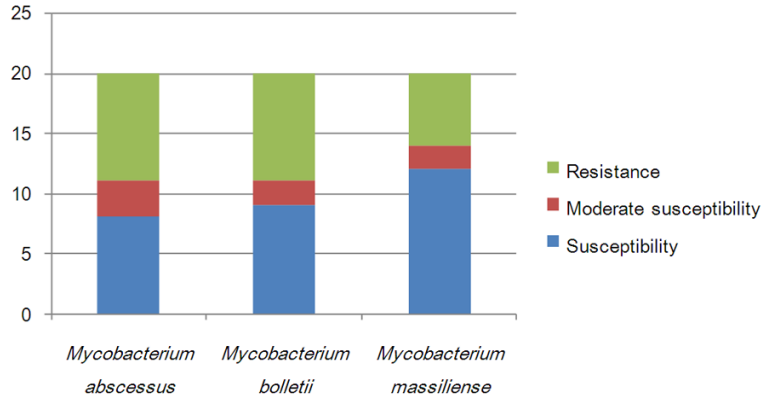

Figure 2. Susceptibility of the *Mycobacterium abscessus* group to 20 selected antibacterial agents.

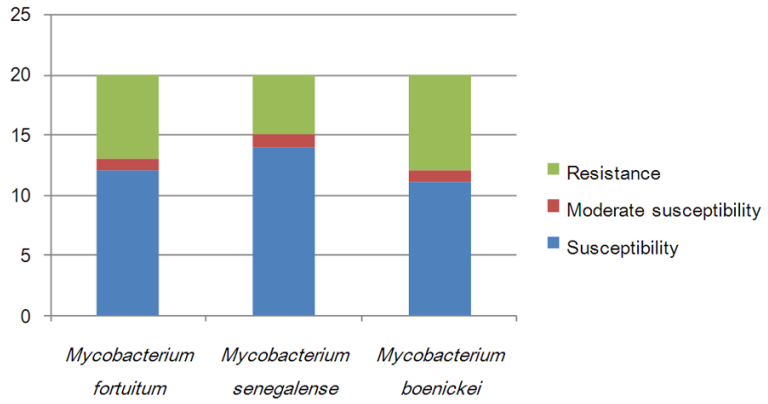

Figure 3. Susceptibility of the *Mycobacterium fortuitum* group to 20 selected antibacterial agents.

susceptible to 11 of the tested agents, and *M. massiliense* was susceptible to 14 (**Figure 2**). *M. fortuitum*, *M. senegalense* and *M. boenickei* were susceptible or moderately susceptible to 12-15 drugs (**Figure 3**), and *M. goodii* and *M. wolinskyi* were susceptible to 14 of the compounds tested (**Figure 4**).

Discussion

In this study, the susceptibility patterns of 40 international reference RGM strains towards 20 selected antimicrobial agents were determined using the CAMH broth microdilution method. To our knowledge, this is the most extensive susceptibility analysis of RGM reference strains to date.

Antimicrobial sensitivity is known to vary across RGM species [1]. In the present study we found that the majority of RGM strains were resistant to the first-line antituberculous agents, with isoniazid displaying the highest MIC, consistent with previous reports [1, 12-14]. Aminoglycosides and fluoroquinolones are the current second-line conventional antituberculous drugs, and amikacin exhibits excellent activity towards *M. abscessus*, *M. chelonae* and *M. fortuitum,* while tobramycin was effective against *M. chelonae* [1, 13, 15-17]. Our results showed that all 40 RGM reference strains were susceptible to amikacin, and 39 strains were susceptible to capreomycin, including *M. chelonae.* Tobramycin was the least active among the aminoglycosides and 9 RGM species were resistant to this drug. In previous studies [1, 15, 16], quinolones were found to be more active against *M. fortuitum* than *M. abscessus*, whereas in the present study, *M. chelonae* was more resistant to quinolones than both *M. fortuitum* and *M. abscessus.* Most strains were more susceptible to Moxifloxacin than they were to the other quinolones, and ofloxacin exhibited the lowest antimycobacterial activity.

Minocycline and doxycycline belong to the tetracycline class of antibiotics, and tigecycline is a derivative of minocycline. In a previous study, minocycline exhibited 50% activity against RGMs, and doxycycline activity was less than 3% [18]. In the present study, *M. chelonae*, *M. abscessus*, *M. bolletii*, *M. massiliense*, *M. fortuitum* and *M. boenickei* were resistant to minocycline, while *M. chelonae*, *M. abscessus*, *M. bolletii* and *M. boenickei* were resistant to doxycycline. In contrast, tigecycline displayed 100% activity and successfully inhibited all 40 RGM strains, consistent with previous reports [19, 20].

Clarithromycin belongs to the macrolide class of antibiotics and this agent displayed good activity against *M. chelonae* and *M. abscessus*

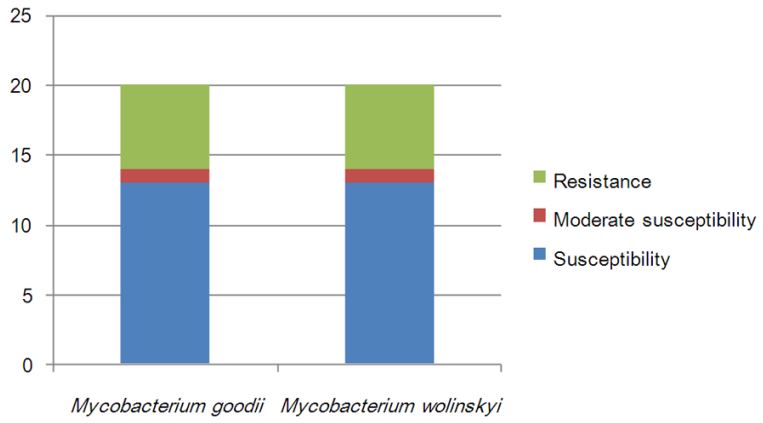

Figure 4. Susceptibility of the *Mycobacterium smegmatis* group to 20 selected antibiotics.

in recent studies [15-17]. However in the present study, *M. boenickei, M. goodie*, *M. wolinskyi and M. flavescens* were resistant to clarithromycin, suggesting these strains may carry the inducible erythromycin methylase gene *erm* that confers macrolide resistance [21], and this should be investigated further in the future.

Cefoxitin and imipenem are important parenteral antibiotics used in the treatment of *M. abscessus* infections [15, 16]. In our study, we used the closely related meropenem instead of imipenem. Cefoxitin and meropenem share the same antibacterial mechanism and operate by inhibiting bacterial cell wall biosynthesis [22]. The *M. abscessus* complex has been divided into three subspecies, namely *M. abscessus*, *M. massiliense* and *M. bolletii* [23, 24]. Our results showed that meropenem displayed a lower MIC value than cefoxitin, and *M. abscessus* and *M. bolletii* were more resistant to these antimicrobial agents than *M. massiliense*. There is increasing evidence that *M. massiliense* is the causative agent of soft tissue infection outbreaks and postsurgery infections [25]. The *M. fortuitum* group [1] has also been divided into three subspecies (*M. fortuitum*, *M. senegalense* and *M. boenickei*). The *M. fortuitum* group has been shown to be susceptible to the majority of antimicrobial agents in previous studies [16, 26]. However, in the present study, *M. fortuitum* was resistant to tobramycin, *M. senegalense* was resistant to the third-line fluoroquinolones ofloxacin, ciprofloxacin and levofloxacin, and *M. boenickei* was more resistant to minocycline and doxycycline than the other two subspecies. The *M. smegmatis* group includes *M. goodii* and *M. wolinskyi* [1], and both these strains were resistant to tobramycin and clarithromycin in our study. The *M. abscessus* complex, *M. chelonae*, the *M. fortuitum* group, *M. mucogenicum* and *M. neoaurum* are the most frequent cause of human infections among the RGMs characterized to date [6]. The results of the present study showed that the *M. abscessus* group, *M. chelonae*, *M. fortuitum, M. boenickei and M. conceptionense* were the most drug-resistant of the RGM strains tested. *M. chelonae* and *M. abscessus* belong to the same complex and share some similar characteristics [27]. In our study, *M. chelonae* proved to be resistant to more drugs than did *M. abscessus*.

In summary, we measured the *in vitro* antimycobacterial activity of 20 selected antibacterial agents against 40 RGM reference strains. The results pave the way for future *in vivo* studies and provide important information for optimizing specific therapies against different RGM species.

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Disclosure of conflict of interest

None.

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