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In vitro susceptibility patterns for slowly growing non-tuberculous mycobacteria in the USA from 2018 to 2022

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Background: Treatment of slowly growing non-tuberculous mycobacteria (SGM) is challenging. *In vitro* antimicrobial susceptibility testing (AST) is needed to optimize a multidrug regimen but requires weeks to result. Aggregated AST patterns, or an antibiogram, of SGM would be helpful to providers.

Objectives: We aggregated and analysed human SGM isolates sent to our laboratory from across the USA between 2018 and 2022 to describe their *in vitro* susceptibility patterns and construct an antibiogram.

Methods: SGM isolates' species/subspecies and mutations in *rrs* or *rrl* were identified by a line probe assay. AST was done primarily by broth microdilution and interpreted using the latest CLSI guideline. Mutational and AST results for SGM with ≥15 isolates were collated and analysed with descriptive statistics.

Results: There were 32 different species/subspecies of SGM from 10131 isolates between January 2018 and December 2022 from across the USA, 80% of which were from organisms in *Mycobacterium avium* complex (MAC). Most specimens were sputum and came from Florida (2892). MAC ranged from 94% to 100% susceptible to clarithromycin, 64% to 91% to amikacin, 2% to 31% to linezolid, and 4% to 41% to moxifloxacin. Non-MAC SGM ranged from 82% to 100% susceptible to clarithromycin, 49% to 100% to amikacin, and 76% to 100% to rifabutin, but susceptibilities to other antimicrobials varied widely. WT *rrs* and *rrl* predicted >96% of phenotypic non-resistance to amikacin and clarithromycin, respectively, whereas mutant genotypes predicted >90% of phenotypic resistance.

Conclusions: Most SGM are likely to be susceptible to clarithromycin and amikacin, complementing their treatment guidance by mycobacterial experts. Molecular identification of resistant genotypes is accurate and helpful. This antibiogram for SGM will help providers.

Introduction

Non-tuberculous mycobacteria (NTM) cause pulmonary and extrapulmonary diseases of considerable morbidity and mortality. Their incidence and prevalence have been rising in the USA.[1] Treatment often requires at least three different antibiotics administered for many months, with significant adverse effects and suboptimal outcomes.[2] Antimicrobial susceptibility testing (AST) is used to select and optimize the therapy but often requires weeks to result. The recent official clinical practice guideline provides evidence-based therapies for the most common NTM, such as *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus*.[2] However, the *in vitro* microbiological data to complement or support these recommendations have not been comprehensively aggregated. Importantly, broth microdilution susceptibility remains the recommended method to guide clinicians on choosing drug regimens for patients with NTM disease.[2–4]

Slowly growing non-tuberculous mycobacteria (SGM) require weeks for growth and AST. Although the recent guideline provides treatment recommendations for some common SGM, an antibiogram is helpful in certain clinical scenarios while

waiting for isolate-specific AST, such as when a patient has intolerance to a guideline-recommended drug or requires timely treatment initiation for an SGM disease. The CLSI recommends that hospitals make antibiograms annually to track changes in susceptibility patterns but requires that at least 30 unique isolates be tested for a specific organism.[5] This is not feasible for most hospitals to perform for mycobacteria given the required laboratory infrastructure and difficulty in accruing enough isolates.

The Mycobacteriology Laboratory at National Jewish Health is a national reference laboratory and receives isolates from across the USA. In addition to AST testing, we used a line probe assay (LPA) and Sanger sequencing to molecularly identify an isolate's species/subspecies and mutations conferring antimicrobial resistance. Using our collection of isolates, we recently published the *in vitro* susceptibility patterns of rapidly growing mycobacteria (RGM).[6] Here, we present the *in vitro* susceptibility patterns, or an antibiogram, for SGM from 2018 through 2022.

Materials and methods

Identification of mycobacterial isolates and detection of drug resistance markers

The SGM were identified by GenoType NTM-DR VER 1.0 line probe (HAIN Lifescience, Nehren, Germany) from primary culture of specimens or subcultured isolates in Mycobacterial Growth Indicator Tubes (MGIT, BD, Franklin Lakes, NJ, USA), 7H10- or 7H11-based agar (Remel, Lenexa, KS, USA) or Lowenstein Jensen media (Remel, Lenexa, KS, USA). This LPA further detected specific mutations conferring constitutive resistance to aminoglycosides (A1408G in the *rrs* gene) and macrolides (A2058C, A2058G, A2059C and A2059G in the *rrl* gene) in MAC isolates.[7] Of note, it could also detect the presence of and mutations in *erm*(41) in *M. abscessus*, which confers inducible macrolide resistance, but SGM do not have this gene.[7] Another identification method was the laboratory-developed Sanger sequencing of a 723 bp *rpoB* region or a 500 bp region of the 16S rRNA gene. The Sanger sequencing procedure was described under the Methods section of our previously published study.[6]

Species and subspecies distinction is based on the List of Prokaryotic Names with Standing in Nomenclature (LPSN).[8] For example, *Mycobacterium intracellulare* subsp. *yongonense* has been proposed by some as equivalent to *M. intracellulare* subsp. *chimaera*[9] as opposed to its own subspecies of *M. intracellulare*.[10] However, it has been treated as a subspecies in this study to adhere to LPSN and evaluate potential variations in susceptibility patterns between these closely related organisms.

AST

The Mycobacteriology Laboratory performed broth microdilution AST for most drugs using lyophilized SLOMYCO and SLOMYCO2 panels (ThermoFisher Scientific, Waltham, MA, USA). The SLOMYCO panel contained the following drugs: amikacin, clarithromycin, ciprofloxacin, doxycycline, ethambutol, isoniazid, linezolid, moxifloxacin, rifabutin, rifampicin, streptomycin and trimethoprim/sulfamethoxazole. This panel was replaced in February 2021 with SLOMYCO2, which contained the same drugs except that ethambutol and isoniazid were removed, and clofazimine, minocycline and a higher concentration of amikacin were added.

Broth microdilution testing was performed in accordance with CLSI guidelines. In brief, after 7–14 days of growth, a 0.5 McFarland suspension was made and diluted in sterile water and then diluted 1:100 in cation-adjusted Mueller–Hinton broth with OADC. These suspensions were used to inoculate 96-well plates containing antimicrobials using the Sensititre AIM Automated Inoculation Delivery System (ThermoFisher Scientific, Waltham, MA, USA). Each plate was then incubated at 36 ± 2°C. *M. avium* (ATCC 700898) served as the quality control organism for each run. Two trained technicians read the MIC for each drug at 7–14 days, in compliance with the CLSI document M24 third edition. If the results did not fall within a 2-fold dilution between the two technicians, a third technician reread the plate or repeated the susceptibility testing for consensus. MIC values were interpreted based on the most recent CLSI guideline, M24S, second edition.[11]

Broth macrodilution was performed for some atypical organisms or organism-drug combinations.[12,13] The colorimetric BACT/Alert 3D Mycobacterial Detection System (bioMérieux, Marcy-l'Etoile, France) was used, which was validated by the laboratory as showing equivalence to the originally described radiometric method. In brief, a 0.5 MacFarland of the control strain (*M. avium* ATCC strain 700898) was inoculated at a 1:100 dilution in Bact/Alert MT culture bottles (bioMérieux) containing no antibiotic for up to 14 days. Test strains were inoculated at a 1:10 dilution into bottles with various antibiotics added in 2-fold dilutions. The MIC for each drug was determined as the lowest drug concentration that inhibits growth of at least 99% of mycobacteria at the time the growth control turned positive. The following organisms were tested with broth macrodilution at various incubation temperatures: *Mycobacterium haemophilum* (32°C), *Mycobacterium xenopi* (42°C), *Mycobacterium kansasii*, *Mycobacterium szulgai*, and azithromycin for MAC (37°C).

Literature review of SGM antimicrobial susceptibility data

We searched PubMed using the following search algorithm: ('antibiogram' [Title/Abstract] OR (('drug'[Title/Abstract] OR 'antibiotic'[Title/Abstract] OR 'antimicrobial'[Title/Abstract]) AND (('susceptibility'[Title/Abstract] OR 'resistance'[Title/Abstract]) AND ('pattern'[Title/Abstract] OR 'testing'[Title/Abstract])))) AND ('nontuberculous mycobacteria'[Title] OR 'NTM'[Title] OR 'nontuberculous mycobacteria'[MeSH Major Topic] OR 'mycobacterium other than tuberculosis'[Title]). We then reviewed each result and included only peer-reviewed studies with a summary of percent susceptible SGM results. We excluded studies without interpretive criteria from CLSI guidelines and without complete species identification of MAC isolates. We then constructed a table with the organism's name, number of isolates tested, country of study's origin, method of identification, method of susceptibility testing and interpretive guideline used. For each organism-antimicrobial combination with two or more studies, we calculated a weighted average based on each study's number of isolates and percent susceptible, excluding our own data. Specifically, this weighted average was the sum of each previous study's percent susceptible for that antimicrobial multiplied by that study's number of isolates tested, then divided by the total number of isolates tested in all previous studies for that antimicrobial. If a study tested fewer than its total number of isolates for a specific antimicrobial, the weighted average was calculated using that actual number of isolates tested for that antimicrobial.

Data acquisition

Human SGM isolates with susceptibilities from January 2018 through December 2022 had their data retrieved and analysed from our laboratory information system (SoftLab, Clearwater, FL, USA). Variables included age, gender, specimen source, state and MIC values. The patient's resident state, if available, served as the isolate's state designation; otherwise, the state of the submitting facility was considered instead. Clinical history and treatment data were not available. Only the isolate corresponding to the first available collection date submitted per patient within this period was included in the analyses.[5] We excluded isolates that were submitted from outside of the USA.

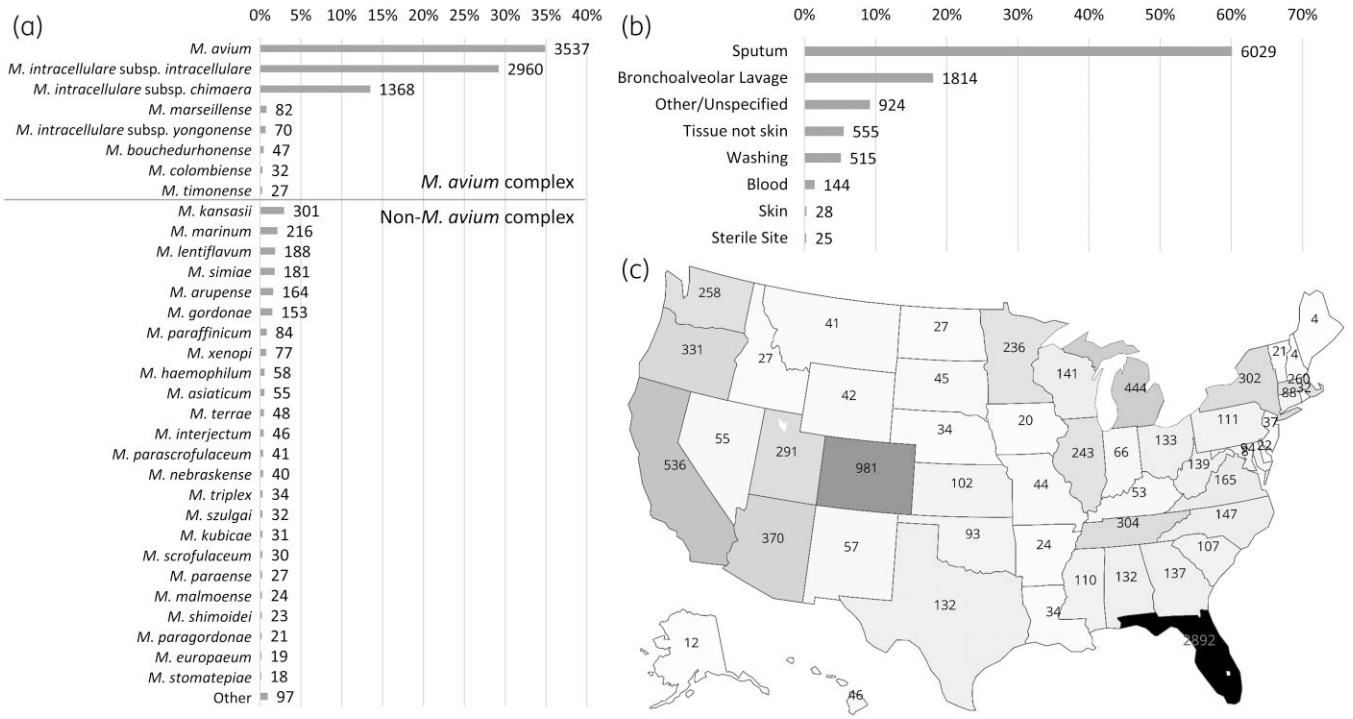

Figure 1. (a) Distribution of slowly growing non-tuberculous mycobacteria (SGM) with ≥15 isolates ($n=10\,131$). Species with <15 isolates were categorized as 'Other SGM'. (b) Distribution of specimen types. (c) Distribution of states from which the isolates came ($n=10\,034$).

Data analysis

An antibiogram representing the percentage of susceptible isolates of a species/subspecies was compiled for SGM with greater than 15 isolates and with complete identification. The 95% CIs for the susceptibility percentages of the antibiogram were calculated using the modified Wald method. An assessment of the susceptibilities for each of the 5 years of the study was also performed for the MAC species with ≥15 isolates for all years. Each antimicrobial drug had MIC_{50} and MIC_{90} values determined by sorting all MIC values from smallest to largest, calculating the cumulative percentage of isolates at each value and determining the lowest MIC that inhibited at least 50% and 90% of the isolates, respectively. The frequencies of specified *rrs* and *rrl* mutations were also evaluated on a subset of MAC isolates where LPA data were available. The exclusion criteria for this analysis were absence of gene detection, mutations with unidentified base changes and presence of heteroresistance, suggestive of a mixed population. The analyses were performed using Pandas (v. 1.5.2) and Numpy (v. 1.21.5) Python libraries.

Ethics

The National Jewish Health Human Research Protection Program reviewed and determined this study (HS-3715) to be of Exempt status. BRANY IRB (EXT21-050-528) reviewed and determined it to meet the waiver criteria per 45 CFR 164.512 (i)2(ii), authorizing it to use and disclose protected health information.

Results

From January 2018 to December 2022, 10 131 isolates of SGM had AST results. There were 32 different species/subspecies of SGM with ≥15 isolates, with MAC as the most common and contributing 80% of all isolates (8123/10 131). Within MAC, the three most common species/subspecies were *M. avium sensu stricto* (3537/8123, 44%), *M. intracellulare* subsp. *intracellulare* (2960/8123, 36%) and *M. intracellulare* subsp. *chimaera* (1368/8123, 17%). The three most common non-MAC SGM were *M. kansasii* (301/10 131, 3%), *Mycobacterium marinum* (216/10 131, 2%) and *Mycobacterium lentiflavum* (188/10 131, 2%) (Figure 1a). The most common specimen type collected was sputum at 60% (6029/10 131) (Figure 1b). Most of the isolates came from patients who resided in Florida (2892), Colorado (981) and California (536) (Figure 1c). The majority of patients were women (61%) (Table S1, available as Supplementary data at *JAC* Online).

The antibiogram showed that, for the 5 year period, all SGM were most susceptible to clarithromycin, with MAC ranging from 94% to 100% and non-MAC 82% to 100%. Amikacin had the second highest susceptibility rates, with MAC ranging from 64% to 91% and non-MAC 49% to 100%. MAC isolates were highly non-susceptible to both linezolid (range 2%–31%) and moxifloxacin (range 4%–41%), but these susceptibility rates were more variable for non-MAC SGM. The non-MAC SGM that were most susceptible to linezolid were *M. haemophilum* (100%), *M. marinum* (96%), *Mycobacterium shimoidei* (94%) and *M. xenopi* (89%); those most susceptible to moxifloxacin were *M. haemophilum* (100%), *M. shimoidei* (90%) and *M. xenopi* (82%). The non-MAC SGM were otherwise generally non-susceptible to other antibiotics except for rifabutin. Most of rifabutin's susceptibility rates ranged from 76% to 100%, excluding *Mycobacterium asiaticum* (59%) and *Mycobacterium simiae* (37%). Its pattern contrasted with that of rifampicin, which was highly variable and generally showed lower susceptibility rates than rifabutin (Figure 2). See Table S2 for 95% CIs.

	Organism	AMK		CLR		LZD		MXF		CIP		RFB		RIF		DOX		MIN		SXT	
		n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
M. avium complex	*M. avium*	3412	65	3404	94	3377	6	3375	27	3364	-	3416	-	3447	-	1603	-	1218	-	1605	-
	M. bouchedurhonense	47	91	46	96	45	31	46	39	46	-	47	-	46	-	30	-	28	-	30	-
	M. colombiense	32	91	32	100	32	22	32	41	32	-	32	-	31	-	19	-	14	-	19	-
	M. intracellulare subsp. *chimaera*	1322	81	1312	98	1299	2	1299	4	1298	-	1315	-	1323	-	603	-	453	-	603	-
	M. intracellulare subsp. *intracellulare*	2916	71	2897	96	2878	2	2877	6	2873	-	2914	-	2919	-	1311	-	948	-	1307	-
	M. intracellulare subsp. *yongonense*	69	64	68	96	66	11	66	20	66	-	68	-	68	-	12	-	9	-	12	-
	M. marseillense	80	82	79	100	79	9	78	15	78	-	80	-	80	-	45	-	36	-	45	-
	M. timonense	27	89	27	100	27	19	27	22	27	-	27	-	27	-	11	-	8	-	11	-
	M. arupense	158	49	157	97	155	9	157	48	157	3	158	97	159	18	85	1	70	3	85	31
	M. asiaticum	54	93	54	100	54	11	54	65	54	2	54	59	55	4	25	0	14	-	25	40
	M. europaeum	18	94	17	100	17	24	17	18	17	0	18	100	18	50	11	-	10	-	11	-
	M. gordonae	144	94	145	99	143	63	144	59	144	15	145	93	145	23	144	14	144	17	144	62
	M. haemophilum	48	100	49	100	48	100	48	100	50	82	50	100	49	100	0	-	0	-	0	-
	M. interjectum	46	87	45	100	45	62	46	61	46	2	46	96	46	59	23	4	17	0	23	61
	M. kansasii	196	90	242	98	214	80	230	65	201	7	192	98	214	63	188	1	189	1	190	48
	M. kubicae	31	94	31	90	31	42	31	71	31	10	31	90	31	19	16	19	10	-	16	50
	M. lentiflavum	184	82	185	100	184	16	184	32	182	15	186	98	185	32	78	5	56	2	79	39
	M. malmoense	24	83	23	96	23	70	23	74	23	30	24	88	23	30	8	-	5	-	8	-
	M. marinum	207	99	208	99	205	96	207	57	207	27	60	100	61	57	208	34	208	37	208	77
	M. nebraskense	38	97	37	100	38	71	37	70	37	46	37	95	38	84	13	-	9	-	13	-
	M. paraense	27	85	27	93	27	70	27	26	27	11	27	85	27	52	15	0	13	-	15	60
	M. paraffinicum	83	89	82	98	82	29	82	33	82	9	83	84	83	61	33	6	30	3	33	33
	M. paragordonae	19	100	20	95	18	44	20	70	20	10	19	84	19	37	19	5	19	0	19	58
	M. parascrofulaceum	39	92	39	95	39	46	39	33	39	0	39	97	39	72	22	0	12	-	22	14
	M. scrofulaceum	29	97	29	86	29	34	29	48	29	7	29	100	29	45	11	-	6	-	11	-
	M. shimoidei	19	95	21	90	16	94	21	90	21	81	17	100	21	24	2	-	2	-	2	-
	M. simiae	156	74	156	82	155	2	155	13	155	1	155	37	156	3	58	0	44	0	59	8
	M. stomatepiae	18	78	18	94	18	33	18	33	18	0	18	83	18	0	7	-	3	-	7	-
	M. szulgai	22	95	26	100	23	65	24	25	26	4	25	76	24	38	21	0	21	5	21	43
	M. terrae	45	56	45	96	44	7	45	40	46	15	46	93	48	12	28	0	17	0	28	43
	M. triplex	34	71	34	100	34	50	34	29	34	3	34	100	34	47	16	0	13	-	16	38
	M. xenopi	62	92	65	98	65	89	68	82	64	41	60	98	62	39	58	5	58	3	58	67

Figure 2. Antibiogram for slowly growing non-tuberculous mycobacteria with ≥15 isolates in the USA from 2018 to 2022. Interpretation was based on the CLSI 2023 M24S guideline. A dash (–) indicates not enough information, no CLSI breakpoint, or number of isolates <15. AMK, amikacin; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; LZD, linezolid; MIN, minocycline; MXF, moxifloxacin; RFB, rifabutin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; %S, percent susceptible.

MAC's susceptibility to clarithromycin and linezolid remained stable over the 5 years, but its susceptibility dropped by >10% to amikacin in the years 2020 and 2022, and >10% to moxifloxacin in 2020, 2021 and 2022 (Figure 3 and Table S3). Tables S4–S6 illustrate the MIC_{50} and MIC_{90} data with notable additions of clofazimine, ethambutol and streptomycin, which did not have CLSI susceptibility breakpoints. For MAC's species/subspecies, rifampicin's MIC_{50} and MIC_{90} values were 1–2 and >4 mg/L, respectively, whereas ethambutol's MIC_{50} and MIC_{90} values were 5 and 10–20 mg/L, respectively. All MIC_{50} and MIC_{90} values for clofazimine were ≤0.25 mg/L.

Among MAC isolates, mutations in *rrs* marking resistance to aminoglycosides[7] were detected only in *M. avium* (1.4%) and *M. intracellulare* subsp. *intracellulare* (0.5%). Meanwhile, mutations in *rrl* marking resistance to macrolides[7] were detected at a higher rate, specifically for *M. avium* (3.2%), *M. intracellulare* subsp. *chimaera* (1.1%), *M. intracellulare* subsp. *intracellulare* (3.5%) and *M. intracellulare* subsp. *yongonense* (8.3%) (Table 1). There were no trends between the years and frequencies of these mutations (Tables S7 and S8). WT *rrs* occurred with 69.7% amikacin-susceptible phenotype on AST, 26.2% intermediate and 4% resistant. Meanwhile, mutant *rrs* occurred with 0% susceptible, 9.4% intermediate and 90.6% resistant. WT *rrl* occurred with 97.4% clarithromycin-susceptible phenotype, 1.3% intermediate and 1.3% resistant. On the other hand, mutant *rrl* occurred with 7.7% susceptible, 0% intermediate and 92.3% resistant (Table 2). Although each species'/subspecies' genotypes generally predicted their susceptibility phenotypes, *M. avium* and *M. intracellulare* subsp. *intracellulare* showed the most discordance (Figures S1 and S2).

Our data were compared with 10 previously published, peer-reviewed studies with SGM *in vitro* susceptibility patterns, shown in Tables 3 and 4. These studies were conducted in either Canada, China, Germany, Greece, Japan, South Korea or the UK. They had various methodologies and interpreted susceptibility based on multiple CLSI guideline editions from 2002 through 2018. This study's percentages of susceptible isolates were similar to the weighted averages of these previous studies for amikacin and clarithromycin but less so for linezolid, moxifloxacin and rifampicin.

Discussion

Complementary to our previous study on RGM,[6] this study reports an antibiogram based on the largest compendium of SGM isolates currently in the USA. This antibiogram can help providers choose empirical regimens while waiting on official AST for their patients' specific SGM isolates. We identified 32 different species/subspecies of SGM from 10131 isolates between January 2018 and December 2022 across the USA, with AST done at National Jewish Health. Most isolates came from Florida, Colorado and California. The most common SGM were MAC, contributing 80%

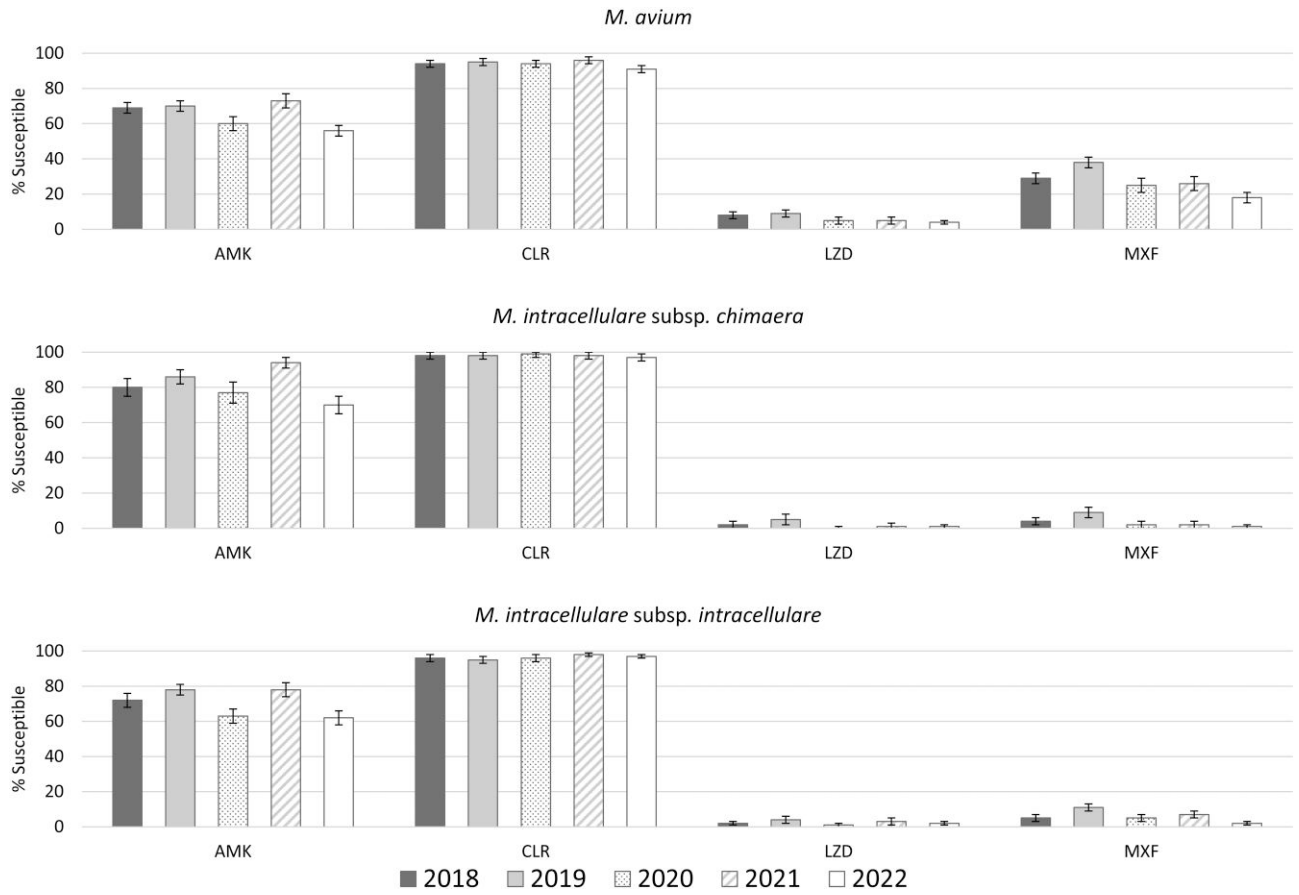

Figure 3. Percentage of *Mycobacterium avium* complex susceptible to amikacin (AMK), clarithromycin (CLR), linezolid (LZD) and moxifloxacin (MXF), stratified by the three most common species/subspecies and years. Error bars indicate the 95% CIs calculated using the modified Wald method.

Table 1. Frequencies of *rrs* mutations, conferring aminoglycoside resistance, and *rrl* mutations, conferring macrolide resistance, in *Mycobacterium avium* complex

		Mutations in *rrs*		Mutations in *rrl*	
Organism	Total	*n*	%	*n*	%
M. avium	1769	25	1.4	56	3.2
M. bouchedurhonense	33	0	0	0	0
M. colombiense	16	0	0	0	0
M. intracellulare subsp. *chimaera*	743	0	0	8	1.1
M. intracellulare subsp. *intracellulare*	1346	7	0.5	47	3.5
M. intracellulare subsp. *yongonense*	36	0	0	3	8.3
M. marseillense	43	0	0	0	0
M. timonense	15	0	0	0	0
Total of *M. avium* complex	4001	32	0.8	114	2.8

of all isolates, in the following order: *M. avium, M. intracellulare* subsp. *intracellulare* and *M. intracellulare* subsp. *chimaera*. Outside of MAC, the most common SGM, in order, were *M. marinum*, *M. kansasii* and *M. lentiflavum*.

Nearly all SGM were susceptible to clarithromycin, followed closely by amikacin. MAC isolates were highly non-susceptible to both linezolid and moxifloxacin, whereas non-MAC isolates had variable susceptibility to them. Otherwise, non-MAC SGM were mostly susceptible to rifabutin *in vitro*, in contrast to rifampicin. Compared with previously published antibiograms worldwide, clarithromycin, amikacin and rifabutin susceptibilities remained reasonably consistent among comparable SGM despite

Table 2. Comparison between *rrs* and *rrl* genotypes and susceptibility phenotypes in *Mycobacterium avium* complex isolates with both mutational analysis and AST

		Susceptibility phenotype against AMK/CLR *n* (%)[a]		
Genotype	*n*/T (%)[b]	S	I	R
WT *rrs* (AMK-S)	3949/3981 (99.2)	2755 (69.7)	1036 (26.2)	158 (4.0)
Mutant *rrs* (AMK-R)	32/3981 (0.8)	0 (0)	3 (9.4)	29 (90.6)
WT *rrl* (CLR-S)	3854/3931 (98.0)	3753 (97.4)	51 (1.3)	50 (1.3)
Mutant *rrl* (CLR-R)	77/3931 (2.0)	6 (7.7)	0 (0)	71 (92.3)

IV AMK's MIC breakpoints: S, ≤16 mg/L; I, 32 mg/L; R, ≥64 mg/L. CLR's MIC breakpoints: S, ≤8 mg/L; I, 16 mg/L; R, ≥32 mg/L. Interpretations based on CLSI 2023 M24S. AMK, amikacin; CLR, clarithromycin; I, intermediate; IV, intravenous; R, resistant; S, susceptible.

[a]*n* (%), frequency of phenotype with percent of frequency of phenotype/total of genotype.

[b]*n*/T (%), frequency of genotype/total tested with percent of this ratio. There were 3981 isolates with both *rrs* and phenotypic AMK susceptibility tested, and 3931 isolates with both *rrl* and phenotypic CLR susceptibility tested.

the different locales, populations and methodologies. Per the 2020 NTM treatment guideline, only *in vitro* susceptibilities to macrolides, amikacin and rifampicin (for *M. kansasii* disease) correlate with clinical outcomes.[2] Our antibiogram thus reassures providers that starting empirical therapy with macrolides and amikacin in SGM disease in the USA is supported by *in vitro* data as part of a multidrug regimen, importantly in combination with ethambutol and a rifamycin, as recommended by the multisociety sponsored NTM guideline.[2]

Rifabutin has been clinically studied in MAC and showed promise as prophylaxis in patients with AIDS[24] and comparable efficacy with rifampicin in multidrug treatment regimens.[25,26] However, rifabutin only has CLSI breakpoints for non-MAC SGM.[4] Literature characterizing the mechanism behind the discordant susceptibility to rifampicin versus rifabutin in SGM is sparse but is likely due to genetic differences in these isolates' *rpoB* genes. This hypothesis is extrapolated from two studies showing that some mutations in *rpoB* of *M. kansasii* and *M. avium* subsp. *paratuberculosis* confer high-level resistance against rifampicin but not rifabutin.[27,28] The guideline and expert consensus address rifabutin as only an alternative to rifampicin as part of the core regimen against specific SGM;[2,3] it has pronounced adverse effects, making providers understandably hesitant to prescribe it instead of rifampicin,[29] but it has fewer and less severe interactions with most other drugs, except notably clarithromycin.[30] Taken together, if tolerated, this study supports the guideline's and consensus statement's view that rifabutin is a valuable alternative to rifamycin in the armamentarium against SGM. However, clinical correlation with rifabutin's *in vitro* susceptibility and the mechanism of differential resistance against rifamycins in SGM should be further investigated.

Ethambutol and rifampicin are notable omissions from our MAC antibiogram considering that they are cornerstone to MAC's three-drug regimen.[2] This is due to CLSI's lack of breakpoints for these drugs against MAC based on some studies suggesting that there was no clinical correlation with their MIC values.[4] However, data from South Korea suggest that having MIC values ≥8 mg/L for both ethambutol and rifampicin is associated with unfavourable outcomes.[31] Our data showed low MIC_{50} values in MAC (ethambutol was 5 mg/L and rifampicin ranged from 1 to 2 mg/L), which may encourage further studies to assess *in vitro* MIC values' correlation with clinical outcomes for these drugs.

The $MIC_{50/90}$ values represent the MIC needed to inhibit 50% or 90% of all the analysed isolates of a specific SGM irrespective of CLSI breakpoint interpretation, with the MIC_{90} being the more stringent measure of *in vitro* potency. As such, it can inform providers or researchers on which antimicrobials to choose for either empirical treatment in clinical care or research on clinical outcomes, respectively. For example, clofazimine exhibited low MIC_{50} and MIC_{90} values against all SGM in our study, but it remains without CLSI breakpoints and an investigational drug against NTM. Its reputation as a drug for NTM was initially tarnished by the 1997 randomized clinical trial by Chaisson *et al.*[32] evaluating the addition of clofazimine to clarithromycin and ethambutol in patients with AIDS and disseminated MAC. The study showed that clofazimine increased mortality, but the results were confounded by the fact the clofazimine arm had 10-fold higher MAC cfu/mL in the blood at baseline compared with the control arm. More recent retrospective studies show that clofazimine is safe, well tolerated and an efficacious substitute for rifamycins in patients with NTM disease. Given these recent observational studies and our data showing low MIC_{50} and MIC_{90} values for clofazimine against all SGM, further breakpoint evaluations and clinical trials on the efficacy of clofazimine against SGM disease are warranted. There is currently an ongoing clinical trial evaluating its efficacy in patients with MAC pulmonary disease (NCT02968212).

Mutational analysis of the *rrs* and *rrl* gene in MAC showed that drug resistance mutations were rare. Mutations in *rrl* (conferring constitutive macrolide resistance) occurred at a higher rate than they did in *rrs* (conferring constitutive aminoglycoside resistance) (2.8% versus 0.8%). One concerning hypothesis is that this was caused by selective pressure from general overuse of azithromycin, the most prescribed antimicrobial in the USA.[33] Macrolides are the workhorse of the three-drug regimen against MAC,[2] and its loss is associated with significantly worse culture conversion rates and mortality.[34–36] Thus, is it imperative that all providers practice antimicrobial stewardship, notably with macrolides.

Among WT *rrs*, only 69.7% were phenotypically amikacin-susceptible but a significant number of isolates were intermediate

Table 3. Comparison of published percent susceptibility data for species and subspecies within *Mycobacterium avium* complex

Organism	Reference	No. of isolates	Country	ID method[a]	AST method[b]	AST interpretation[c]	% Susceptible reported			
							AMK	CLR	LZD	MXF
M. avium	Cho 2018[14]	1060	South Korea	3	I	B	52.6	94.4	24.7[d]	22.6
	Andrews 2020[15]	212	Canada	—	I	C	43.5	90.1	10.4	40
	Uchiya 2018[16]	76	Japan	4	VI	D	77.6	94.7	—	—
	Wetzstein 2020[17]	62	Germany	8	I	C	85.5	98.4	3.2	12.9
	Li 2022[18]	52	China	2, 3, 4, 6, 7	I	B	78.9	96.2	10	23.1
	Wei 2015[19]	50	China	1, 2, 7	II	C[e]	80	94	54	70
	Gitti 2011[20]	20	Greece	1, 8	V	A	—	85	0	40
	$\bar{x}$						55.7	93.9	21.5	26.6
	This study	3412	USA	3, 8	I	C	65	94[d]	6[d]	27[d]
M. colombiense	Li 2022[18]	14	China	2, 3, 4, 6, 7	I	B	64.2	85.7	21.4	7.1
	This study	32	USA	3, 8	I	C	91	100	22	41
M. intracellulare subsp. *chimaera*	Li 2022[18]	22	China	2, 3, 4, 6, 7	I	B	81.8	77.3	13.6	22.7
	Wetzstein 2020[17]	18	Germany	8	I	C	94.4	100	16.7	16.7
	$\bar{x}$						87.5	87.5	15	20
	This study	1316	USA	3, 8	I	C	81	98[d]	2[d]	4[d]
M. intracellulare (no subspecies identification)	Cho 2018[14]	823	South Korea	3	I	B	57.7	94.2	8.5[d]	5.5
	Li 2022[18]	165	China	2, 3, 4, 6, 7	I	B	72.1	95.2	15.2	13.9
	Zhao 2014[21]	52	China	1, 3, 4	III	C[e]	69.2	82.7	0	17.3
	Andrews 2020[15]	50	Canada	—	I	C	73.3	96	2	0
	Gitti 2011[20]	5	Greece	1, 8	V	A	—	100	20	20
	Wetzstein 2020[17]	5	Germany	8	I	C	80	100	40	0
	$\bar{x}$						61.2	93.9	9.1	7.1
	This study[f]	2916	USA	3, 8	I	C	71	96[d]	2[d]	6[d]
M. marseillense	Li 2022[18]	25	China	2, 3, 4, 6, 7	I	B	80	96	20	16
	This study	80	USA	3, 8	I	C	82	100[d]	9[d]	15[d]

A dash (—) indicates information not reported, available or able to be calculated. AMK, amikacin; AST, antimicrobial susceptibility testing; CLR, clarithromycin; ID, identification; LZD, linezolid; MXF, moxifloxacin; $\bar{x}$, weighted average (excludes this study).

[a]*ID methods:* 1, biochemical tests; 2, 16S rRNA sequencing; 3, *rpoB* sequencing; 4, *hsp65* sequencing; 5, Internal Transcribed Spacer (ITS) sequencing; 6, MALDI-TOF MS; 7, 16S–23S spacer region sequencing; 8, line probe assay.

[b]*AST methods:* I, broth microdilution, Sensititre Myco SLOMYCO plates; II, broth microdilution, house-made plates; III, broth microdilution, house-made plates + 0.02% Tween; IV, broth macrodilution, Bactec 460 and Bactec MGIT 960 EPICenter; V, Etest, AB BioDisk, read at 5–10 days; VI, Broth MIC NTM system (Kyokuto Pharmaceutical Industrial Co.).

[c]*AST interpretations:* A, M24-A CLSI 2003; B, M24-A2 CLSI 2011; C, M62 CLSI 2018 or M24S CLSI 2023; D, Broth MIC NTM System Manual (Kyokuto Pharmaceutical Industrial Co.), but percent susceptible was recalculated based on MICs presented in the study.

[d]Fewer than the study's total number of isolates were tested for this antimicrobial.

[e]The percent susceptible was manually calculated by enumerating the study's number of isolates per MIC value and then interpreted based on CLSI 2023 M24S.

[f]*M. intracellulare* subsp. *intracellulare* was included into this group.

(26.2%)—only 4% were actually resistant. Together, WT *rrs* predicted non-resistance (susceptible and intermediate) in 96% of MAC isolates, suggesting that amikacin is still a viable option, administered either IV or inhalationally, especially because the latter achieves a much higher drug concentration inside the lungs.[37–39] Meanwhile, mutant *rrs* decently predicted phenotypic amikacin resistance, as 90.6% of these MAC isolates were resistant and none were susceptible. WT *rrl* was highly predictive of the clarithromycin-susceptible phenotype at 97%, whereas its mutant counterpart was predictive of the clarithromycin-resistant phenotype at 92.3%. Overall, the NTM-DR VER 1.0 LPA for mutational analysis of *rrs* and *rrl* results faster than phenotypic AST, is highly predictive of phenotypic amikacin and clarithromycin susceptibility, but correlates less well with phenotypic testing than previously published by other studies.[7,40] Notably, 9.4% of mutant *rrs* isolates were amikacin-intermediate, and 7.7% of mutant *rrl* isolates were clarithromycin-susceptible; expert consultation is recommended to discuss the use of amikacin or clarithromycin in these cases. These nuances regarding genotype and phenotype illustrate the usefulness of performing both mutational analysis and phenotypic AST as opposed to just one or the other.

Further, there were noticeable variations in both the genotypic and phenotypic susceptibility patterns among the MAC species/subspecies. *M. avium*, *M. intracellulare* subsp. *intracellulare* and

Table 4. Comparison of published percent susceptibility data for non-tuberculous slowly growing mycobacteria other than *Mycobacterium avium* complex

							% Susceptible reported										
							CLSI 2018–2023									CLSI 2011	CLSI 2003
Organism	Reference	No. of isolates	Country	ID method[a]	AST method[b]	AST interpretation[c]	AMK	CLR	LZD	MXF	CIP	RFB	RIF	DOX	SXT	EMB	INH
M. gordonae	Gitti 2011[20]	2	Greece	1, 8	V	A	100	100	100	100	100	—	100	—	100	100	50
	This study	145	USA	3, 7, 8	I	C	94[d]	99	63[d]	59[d]	15[d]	93	23	14[d]	62[d]	—	—
M. kansasii	Cowman 2016[22]	181	UK	1, 8	IV	B	99	87	—	—	94	—	94	—	—	86	0
	Liu 2021[23]	31	China	2, 3, 4, 5, 6	I	C	96.8	100	96.8	96.8	32.3	96.8	93.6	9.7	48.4	—	—
	Gitti 2011[20]	10	Greece	1, 8	V	A	100	100	100	100	80	—	100	—	100	100	70
	x̄						98.7	89.4	97.6	97.6	83.5	—	94.2	—	61	86.7	4.3
	This study	242	USA	3, 7, 8	I	C	90[d]	97	80[d]	66[d]	6[d]	98[d]	64[d]	1[d]	49[d]	—	—
M. malmoense	Cowman 2016[22]	49	UK	1, 8	IV	B	90	96	—	—	59	—	67	—	—	96	0
	This study	24	USA	3, 7, 8	I	C	83	96[d]	70[d]	74[d]	30[d]	88	30[d]	—	—	—	—
M. marinum	Gitti 2011[20]	2	Greece	1, 8	V	A	100	100	50	50	50	—	0	—	100	100	0
	This study	208	USA	3, 7, 8	I	C	99[d]	99	96[d]	57[d]	27[d]	100[d]	57[d]	34	77	—	—
M. scrofulaceum	Gitti 2011[20]	2	Greece	1, 8	V	A	100	100	100	0	0	—	100	—	—	100	0
	This study	29	USA	3, 7, 8	I	C	97	86	34	48	7	100	45	—	—	—	—
M. simiae	Cowman 2016[22]	57	UK	1, 8	IV	B	89[d]	19	—	—	64[d]	—	5.5[d]	—	—	20[d]	0[d]
	This study	156	USA	3, 7, 8	I	C	74	82	2[d]	13[d]	1[d]	37[d]	3	0[d]	8[d]	—	—
M. xenopi	Cowman 2016[22]	219	UK	1, 8	IV	B	100	100	—	—	98	—	99	—	—	48	57
	Andrews 2020[15]	19	Canada	—	I	C	100	100	—	86	100	100	74	—	—	—	—
	Gitti 2011[20]	1	Greece	1, 8	V	A	100	100	0	100	100	—	100	—	100	100	0
	x̄						100	100	—	86.7	98.2	—	97	—	—	100	56.7
	This study	68	USA	3, 7, 8	I	C	93[d]	98[d]	88[d]	87	39[d]	98[d]	38[d]	4[d]	65[d]	—	—

A dash (—) indicates information not reported, available or able to be calculated. AMK, amikacin; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; EMB, ethambutol; ID, identification; INH, isoniazid; LZD, linezolid; MXF, moxifloxacin; RFB, rifabutin; RIF, rifampicin; SXT, trimethoprim/sulfamethoxazole; x̄, weighted average (excludes this study).

[a]*ID methods:* 1, biochemical tests; 2, 16S rRNA sequencing; 3, *rpoB* sequencing; 4, *hsp65* sequencing; 5, ITS sequencing; 6, MALDI-TOF MS; 7, 16S–23S spacer region sequencing; 8, line probe assay.

[b]*AST methods:* I, broth microdilution, Sensititre Myco SLOMYCO plates; II, broth microdilution, house-made plates; III, broth microdilution, house-made plates + 0.02% Tween; IV, broth macrodilution, Bactec 460 and Bactec MGIT 960 EPICenter; V, Etest, AB BioDisk; VI, Broth MIC NTM system (Kyokuto Pharmaceutical Industrial Co.).

[c]*AST interpretations:* A, M24-A CLSI 2003; B, M24-A2 CLSI 2011; C, M62 CLSI 2018 or M24S CLSI 2023; D, Broth MIC NTM System Manual (Kyokuto Pharmaceutical Industrial Co.), but percent susceptible was recalculated based on the frequencies of MIC values presented in the study.

[d]Fewer than the study's total number of isolates were tested for this antimicrobial.

M. intracellulare subsp. *yongonense* had 10%–20% lower rates of susceptibility to amikacin compared with other species/subspecies. Mutations in *rrs* and *rrl*, along with varying concordances with phenotypic susceptibility, were found only in *M. avium*, *M. intracellulare* subsp. *chimaera*, *M. intracellulare* subsp. *intracellulare* and *M. intracellulare* subsp. *yongonense*. These variations observed may be due to the significantly larger sample sizes of these species/subspecies, technical variation in laboratory testing and AST plates, or by actual differences in their microbiological or epidemiological characteristics. Therefore, MAC species/subspecies identification should be done for both clinical and investigational reasons.

This study has notable limitations and strengths. A recent study out of Europe promotes the establishment of epidemiological cut-off values (ECVs) for NTM.[41] Our study's first limitation is that it used data from a single centre only and therefore could not appropriately establish ECVs for SGM in the USA per CLSI criteria.[42] However, our primary objective was to use established CLSI breakpoints to create an antibiogram, defined as a report of analysed AST profiles at a single institution over a specific time period.[5]

Another limitation is that regional or state-level susceptibility patterns cannot be observed because we amalgamated the data from all the states in the USA. Further, this study could not distinguish isolates from treatment-naive versus treatment-experienced patients. Because ours is a referral laboratory for AST and centre for refractory NTM disease management, the patients are likely to be treatment-experienced and have MDR strains, potentially overrepresenting the resistance rates for first-line antimycobacterials. Because we included only the first index isolate of a specific SGM per patient, we could not capture the impact of heteroresistance in SGM. Finally, this study could not exclude one-off results that were incorrect (i.e. needed to be repeated, runs where quality control did not pass, or runs with manufactured lots that gave unusual results). However, the overall sample size is large enough to mitigate this limitation.

In fact, this study's key strength is that it has the largest sample size of SGM isolates coming from all 50 states in the USA, helping with its generalizability. Furthermore, the laboratory is highly experienced and has a robust infrastructure dedicated to NTM AST, ensuring general consistency and precision of AST results.

In summary, this comprehensive antibiogram of SGM in the USA is an invaluable tool for providers and complements the recent NTM treatment guideline, the consensus recommendations for less common NTM, and our RGM antibiogram.[2,3,6] Barring the few exceptions, *in vitro* AST remains key to tailoring a multidrug regimen against NTM, and this antibiogram can help providers start timely empirical regimens for SGM while waiting for their isolate-specific susceptibilities. Molecular identification of MAC species/subspecies and mutations conferring constitutive amikacin and clarithromycin resistance should be done in addition to phenotypic AST. Our antibiogram should supplement, but not supplant, clinical judgement, patient-centred care and advice from mycobacterial experts.

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Transparency declarations

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Supplementary data

Figures S1 and S2 and Tables S1 to S8 are available as Supplementary data at *JAC* Online.

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