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SUSCEPTIBILITY



# Drug Susceptibility Distributions of *Mycobacterium chimaera* and Other Nontuberculous Mycobacteria

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**ABSTRACT** Recent outbreaks of cardiac surgery-associated *Mycobacterium chimaera* infections have highlighted the importance of species differentiation within the *Mycobacterium avium* complex and pointed to a lack of antibiotic susceptibility data for *M. chimaera*. Using the MGIT 960/EpiCenter TB eXiST platform, we have determined antibiotic susceptibility patterns of 48 clinical *M. chimaera* isolates and 139 other nontuberculous mycobacteria, including 119 members of the *M. avium* complex and 20 *Mycobacterium kansasii* isolates toward clofazimine and other drugs used to treat infections with slow-growing nontuberculous mycobacteria (NTM). $MIC_{50}$, $MIC_{90}$, and tentative epidemiological cutoff (ECOFF) values for clofazimine were 0.5 mg/liter, 1 mg/liter, and 2 mg/liter, respectively, for *M. chimaera*. Comparable values were observed for other *M. avium* complex members, whereas lower $MIC_{50}$ (≤0.25 mg/liter), $MIC_{90}$ (0.5 mg/liter), and ECOFF (1 mg/liter) values were found for *M. kansasii*. Susceptibility to clarithromycin, ethambutol, rifampin, rifabutin, amikacin, moxifloxacin, and linezolid was in general similar for *M. chimaera* and other members of the *M. avium* complex, but increased for *M. kansasii*. The herein determined MIC distributions, $MIC_{90}$, and ECOFF values of clofazimine for *M. chimaera* and other NTM provide the basis for the definition of clinical breakpoints. Further studies are needed to establish correlation of *in vitro* susceptibility and clinical outcome.

**KEYWORDS** *Mycobacterium chimaera*, *Mycobacterium avium* complex, drug susceptibility testing, clofazimine, resistance, antibiotic resistance

*Mycobacterium chimaera* is a slow-growing nontuberculous mycobacterium (NTM) that was established in 2004 as a new species within the *Mycobacterium avium* complex (1). In the past, the number of infections with *M. chimaera* was underestimated, as commercial mycobacterial identification systems such as line probe assays failed to identify *M. chimaera* to species level and thus classified *M. chimaera* as *M. avium* complex, *M. avium*, or *Mycobacterium intracellulare* (1, 2). *M. chimaera* is differentiated from other members of the *M. avium* complex by a unique 16S rRNA gene sequence and internal transcribed spacer (ITS) region (1). Recently, a global outbreak of cardiac surgery-associated *M. chimaera* infections highlighted the importance of species identification within the *M. avium* complex (3, 4). The outbreak was linked to contaminated water reservoirs of heater-cooler devices that spread *M. chimaera* by aerosols during open chest surgery (5, 6). Severe, disseminated *M. chimaera* infections with a high case fatality rate were observed (7).

Due to the limited ability of commercial identification methods to adequately identify *M. chimaera*, few studies have reported drug susceptibility data on *M. chimaera*. Recent studies analyzed antimicrobial susceptibility of *M. chimaera* using a commercial microdilution system, the SLOWMYCO Sensititre panel from Trek Diagnostic Systems, and reported similar susceptibility patterns for *M. chimaera* as for other members of the *M. avium* complex (8–11). Recommended treatment options for disseminated *M.*

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**TABLE 1** Number and origin of NTM isolates included in this study

| Species[a] | No. (%) of respiratory isolates | No. (%) of nonrespiratory isolates | No. (%) of isolates with unknown origin | Total |
|---|---|---|---|---|
| *M. avium* (MAC) | 55 (69) | 8 (10) | 17 (21) | 80 |
| *M. intracellulare* (MAC) | 30 (97) | 0 (0) | 1 (3) | 31 |
| *M. chimaera* (MAC) | 34 (71) | 13 (27) | 1 (2) | 48 |
| Other MAC | 6 (75) | 1 (12.5) | 1 (12.5) | 8 |
| *M. kansasii* | 12 (60) | 5 (25) | 3 (15) | 20 |

[a]MAC, *M. avium* complex.

*chimaera* infections include combination therapy with macrolides, rifamycins, ethambutol, amikacin, and clofazimine (7). Clofazimine is not yet included in the SLOWMYCO Sensititre antibiotic panel and consequently clofazimine MIC data for *M. chimaera* are scarce. We have previously established automated quantitative drug susceptibility testing (DST) for slow-growing NTM using the MGIT 960/EpiCenter TB eXiST platform (12, 13). We here report on MIC distributions of clofazimine and other drugs used to treat NTM infections for 48 clinical *M. chimaera* isolates and 139 other nontuberculous mycobacteria, including 119 members of the *M. avium* complex and 20 *Mycobacterium kansasii* isolates.

## RESULTS

***M. chimaera* clofazimine MIC distribution.** MICs of clofazimine were determined for 48 clinical, nonduplicate *M. chimaera* isolates using the MGIT 960/EpiCenter TB eXiST system (Becton Dickinson, Sparks, MD). A clofazimine concentration range of 0.25 mg/liter to 4 mg/liter was tested in 2-fold serial dilutions. Out of 48 *M. chimaera* isolates, 34 (71%) were of respiratory origin and 13 (27%) isolates were of nonrespiratory origin (Table 1). For one isolate, the source was unknown. Clofazimine MIC values for *M. chimaera* ranged from ≤0.25 mg/liter to 2 mg/liter (Fig. 1A, Table 2). $MIC_{50}$ and $MIC_{90}$ values of 0.5 mg/liter and 1 mg/liter were determined. A tentative epidemiological cutoff (ECOFF) was set at 2 mg/liter by visual inspection of the MIC distribution (Fig. 1). The clofazimine MIC distribution of *M. chimaera* was compared to MIC distributions of 119 *M. avium* complex isolates, including *M. avium* ($n = 80$), *M. intracellulare* ($n = 31$), *Mycobacterium yongonense* ($n = 3$), *Mycobacterium timonense* ($n = 2$), *Mycobacterium bouchedurhonense* ($n = 1$), *Mycobacterium colombiense* ($n = 1$), and *Mycobacterium vulneris* ($n = 1$) (Fig. 1B to E, Table 2). The MIC range, $MIC_{50}$, $MIC_{90}$, and tentative ECOFF values of *M. chimaera* and other *M. avium* complex isolates were comparable. The clofazimine MIC distribution of *M. kansasii*, a slow-growing nontuberculous mycobacterium not related to the *M. avium* complex, showed lower $MIC_{50}$ (≤0.25 mg/liter), $MIC_{90}$ (0.5 mg/liter), and tentative ECOFF value (1 mg/liter) compared to *M. chimaera* and other *M. avium* complex species (Fig. 1F).

**Susceptibility distributions of additional drugs used for the treatment of *M. chimaera* infections.** Susceptibility patterns of additional drugs used for the treatment of *M. chimaera* infections, such as clarithromycin, ethambutol, rifampin, rifabutin, amikacin, moxifloxacin, and linezolid, are shown in Fig. 2 and Table 2 for *M. chimaera*, *M. avium* complex species, and *M. kansasii*. Susceptibility to these drugs was in general comparable for *M. chimaera* and other members of the *M. avium* complex. Lower MIC values were observed for *M. kansasii* toward amikacin, linezolid, moxifloxacin, rifampin, and rifabutin compared to *M. chimaera* and the *M. avium* complex.

**Macrolide and amikacin resistance.** For two *M. avium* isolates and one isolate each of *M. chimaera* and *M. intracellulare*, MIC values of ≥32 mg/liter were observed for clarithromycin, which indicates macrolide resistance according to CLSI guidelines (14). Sequence analysis of the 23S rRNA gene of both *M. avium* isolates and *M. intracellulare* revealed mutations at nucleotide position A2059G (*E. coli* numbering), thereby providing a genotypic confirmation of the high-level macrolide-resistance phenotype. However, for the *M. chimaera* isolate, no mutation could be detected at nucleotide positions A2058/A2059. Repeated clarithromycin testing confirmed the decreased *in vitro* macrolide susceptibility of this isolate that was observed after prolonged macrolide treatment. Two *M. avium* and two *M. intracellulare* isolates exhibited MIC values of

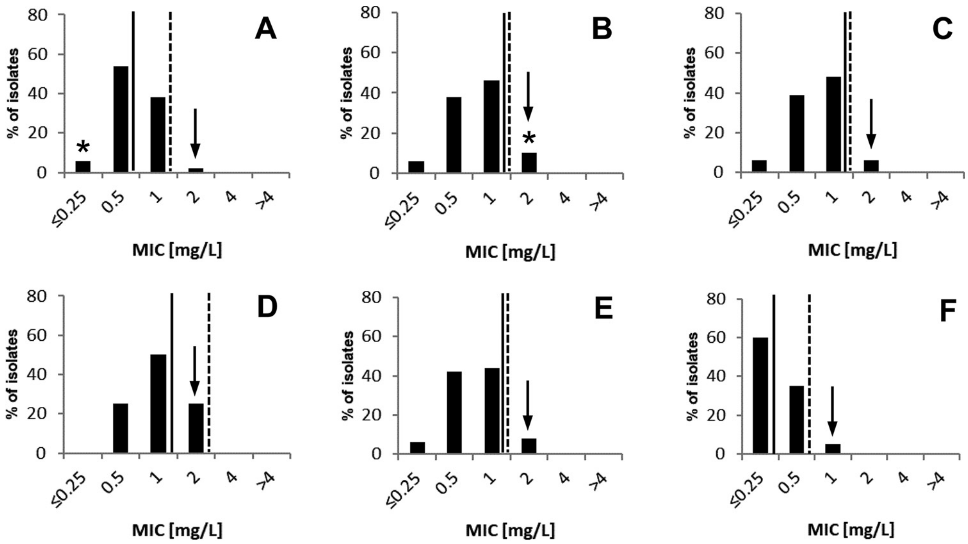


**FIG 1** MIC distributions of clofazimine for *M. chimaera* ($n = 48$) (A), *M. avium* ($n = 80$) (B), *M. intracellulare* ($n = 31$) (C), other MAC ($n = 8$) (D), *M. avium* complex overall ($n = 167$) (E), and *M. kansasii* ($n = 20$) (F). Tentative ECOFF (arrow), $MIC_{50}$ (solid line), and $MIC_{90}$ (dashed line) are indicated. The clofazimine MIC values of the type strains *M. avium* ATCC 19421 and *M. chimaera* DSM 44623 are indicated (*).

≥20 mg/liter for amikacin. One *M. intracellulare* isolate exhibited an A1408G mutation in the 16S rRNA gene (*E. coli* numbering), which is known to confer high-level aminoglycoside resistance (15, 16). In contrast, the second *M. intracellulare* isolate and the two *M. avium* isolates carried a wild-type 16S rRNA allele. Therefore, the molecular mechanisms underlying decreased susceptibility in these strains remain elusive.

## DISCUSSION

Treatment of *M. chimaera* and *M. avium* complex infections is complicated and requires multidrug regimens. Treatment options are limited, especially for macrolide-resistant isolates (7). Clofazimine, a drug traditionally used in leprosy therapy and recently recommended by the World Health Organization (WHO) for the treatment of multidrug-resistant tuberculosis (MDR-TB), is also increasingly used to treat severe *M. avium* complex infections (17, 18). Elevated MICs for clofazimine have been reported for *M. avium* and *M. intracellulare* and suggest the occurrence of resistant isolates (19). Whereas for *Mycobacterium tuberculosis* complex the WHO has released guidelines on clofazimine susceptibility testing and defined clinical breakpoints, i.e., critical concentrations, to separate resistant from susceptible isolates, such guidelines are lacking for NTM (20). Determination of MIC distributions and ECOFFs is a prerequisite for the assignment of clinical breakpoints.

Clofazimine MIC distribution data have to our knowledge not yet been reported for *M. chimaera*. Pang et al. reported a MIC of 0.5 mg/liter for clofazimine for the type strain *M. chimaera* DSM 44623 (21). We determined the $MIC_{50}$, $MIC_{90}$, and ECOFF values to be 0.5 mg/liter, 1 mg/liter, and 2 mg/liter, respectively, based on the MIC distribution of 48 clinical isolates of *M. chimaera* using the MGIT 960/EpiCenter TB eXiST platform and showed that these values are comparable to clofazimine $MIC_{50}$, $MIC_{90}$, and ECOFF values of other members of the *M. avium* complex, including *M. avium sensu stricto* and *M. intracellulare*. Our data are in agreement with different reports of clofazimine susceptibility data for *M. avium* complex (19, 22–24). A $MIC_{50}$ of 1 mg/liter for *M. avium* complex was found by van Ingen et al. (24), and $MIC_{90}$ values of 4 mg/liter and 1 mg/liter were described by Huang et al. for *M. avium* and *M. intracellulare*, respectively (23). Luo et al. determined a clofazimine ECOFF of 2 mg/liter for *M. avium* and *M. intracellulare* (19). The clofazimine MIC distribution of *M. kansasii* was shifted toward lower MICs

**TABLE 2** Assignment of NTM isolates to susceptibility categories in the MGIT 960 system

| | | *In vitro* DST category of isolates (*n*)[b] | | | |
|---|---|---|---|---|---|
| **Drug/species[a]** | **MIC (mg/liter)** | **S** | **I** | **R** | **No. of isolates** |
| Clarithromycin | | | | | |
| *M. avium* | 4 | 67 | 11 | 2 | 80 |
| | 16 | 78 | 0 | 2 | |
| | 32 | 78 | 0 | 2 | |
| | 64 | 78 | 0 | 2 | |
| *M. chimaera* | 4 | 47 | 0 | 1 | 48 |
| | 16 | 47 | 0 | 1 | |
| | 32 | 47 | 1 | 0 | |
| | 64 | 48 | 0 | 0 | |
| *M. intracellulare* | 4 | 29 | 1 | 1 | 31 |
| | 16 | 30 | 0 | 1 | |
| | 32 | 30 | 0 | 1 | |
| | 64 | 31 | 0 | 0 | |
| Other MAC | 4 | 8 | 0 | 0 | 8 |
| | 16 | 8 | 0 | 0 | |
| | 32 | 8 | 0 | 0 | |
| | 64 | 8 | 0 | 0 | |
| *M. kansasii* | 4 | 20 | 0 | 0 | 20 |
| | 16 | 20 | 0 | 0 | |
| | 32 | 20 | 0 | 0 | |
| | 64 | 20 | 0 | 0 | |
| Rifampin | | | | | |
| *M. avium* | 1 | 2 | 16 | 62 | 80 |
| | 4 | 25 | 32 | 23 | |
| | 20 | 60 | 18 | 2 | |
| *M. chimaera* | 1 | 9 | 14 | 25 | 48 |
| | 4 | 28 | 19 | 1 | |
| | 20 | 48 | 0 | 0 | |
| *M. intracellulare* | 1 | 1 | 2 | 28 | 31 |
| | 4 | 3 | 26 | 2 | |
| | 20 | 30 | 1 | 0 | |
| Other MAC | 1 | 4 | 1 | 3 | 8 |
| | 4 | 5 | 3 | 0 | |
| | 20 | 8 | 0 | 0 | |
| *M. kansasii* | 1 | 19 | 0 | 1 | 20 |
| | 4 | 20 | 0 | 0 | |
| | 20 | 20 | 0 | 0 | |
| Rifabutin | | | | | |
| *M. avium* | 0.1 | 11 | 15 | 54 | 80 |
| | 0.4 | 39 | 34 | 7 | |
| | 2 | 78 | 0 | 2 | |
| *M. chimaera* | 0.1 | 9 | 7 | 32 | 48 |
| | 0.4 | 36 | 10 | 2 | |
| | 2 | 47 | 1 | 0 | |
| *M. intracellulare* | 0.1 | 0 | 1 | 30 | 31 |
| | 0.4 | 10 | 19 | 2 | |
| | 2 | 31 | 0 | 0 | |
| Other MAC | 0.1 | 3 | 2 | 3 | 8 |
| | 0.4 | 5 | 3 | 0 | |
| | 2 | 8 | 0 | 0 | |
| *M. kansasii* | 0.1 | 20 | 0 | 0 | 20 |
| | 0.4 | 20 | 0 | 0 | |
| | 2 | 20 | 0 | 0 | |
| Ethambutol | | | | | |
| *M. avium* | 5 | 46 | 19 | 15 | 80 |
| | 12.5 | 71 | 4 | 5 | |
| | 50 | 78 | 0 | 2 | |

(Continued on next page)

**TABLE 2** (Continued)

| Drug/species[a] | MIC (mg/liter) | *In vitro* DST category of isolates (*n*)[b] | | | No. of isolates |
|---|---|---|---|---|---|
| | | S | I | R | |
| *M. chimaera* | 5 | 19 | 4 | 25 | 48 |
| | 12.5 | 47 | 1 | 0 | |
| | 50 | 48 | 0 | 0 | |
| *M. intracellulare* | 5 | 27 | 2 | 2 | 31 |
| | 12.5 | 30 | 0 | 1 | |
| | 50 | 31 | 0 | 0 | |
| Other MAC | 5 | 7 | 1 | 0 | 8 |
| | 12.5 | 8 | 0 | 0 | |
| | 50 | 8 | 0 | 0 | |
| *M. kansasii* | 5 | 19 | 0 | 1 | 20 |
| | 12.5 | 20 | 0 | 0 | |
| | 50 | 20 | 0 | 0 | |
| Amikacin | | | | | |
| *M. avium* | 1 | 0 | 0 | 80 | 80 |
| | 4 | 5 | 27 | 48 | |
| | 20 | 72 | 6 | 2 | |
| *M. chimaera* | 1 | 0 | 1 | 47 | 48 |
| | 4 | 18 | 14 | 16 | |
| | 20 | 48 | 0 | 0 | |
| *M. intracellulare* | 1 | 1 | 0 | 30 | 31 |
| | 4 | 3 | 11 | 17 | |
| | 20 | 28 | 1 | 2 | |
| Other MAC | 1 | 3 | 0 | 5 | 8 |
| | 4 | 4 | 2 | 2 | |
| | 20 | 8 | 0 | 0 | |
| *M. kansasii* | 1 | 3 | 0 | 17 | 20 |
| | 4 | 20 | 0 | 0 | |
| | 20 | 20 | 0 | 0 | |
| Moxifloxacin | | | | | |
| *M. avium* | 0.5 | 29 | 26 | 25 | 80 |
| | 2.5 | 75 | 3 | 2 | |
| | 10 | 79 | 1 | 0 | |
| *M. chimaera* | 0.5 | 11 | 24 | 13 | 48 |
| | 2.5 | 48 | 0 | 0 | |
| | 10 | 48 | 0 | 0 | |
| *M. intracellulare* | 0.5 | 3 | 8 | 20 | 31 |
| | 2.5 | 30 | 1 | 0 | |
| | 10 | 31 | 0 | 0 | |
| Other MAC | 0.5 | 3 | 1 | 4 | 8 |
| | 2.5 | 7 | 0 | 1 | |
| | 10 | 8 | 0 | 0 | |
| *M. kansasii* | 0.5 | 20 | 0 | 0 | 20 |
| | 2.5 | 20 | 0 | 0 | |
| | 10 | 20 | 0 | 0 | |
| Linezolid | | | | | |
| *M. avium* | 1 | 1 | 1 | 78 | 80 |
| | 4 | 8 | 8 | 64 | |
| | 16 | 27 | 34 | 19 | |
| *M. chimaera* | 1 | 0 | 2 | 46 | 48 |
| | 4 | 3 | 13 | 32 | |
| | 16 | 40 | 8 | 0 | |
| *M. intracellulare* | 1 | 0 | 1 | 30 | 31 |
| | 4 | 2 | 5 | 24 | |
| | 16 | 12 | 14 | 5 | |
| Other MAC | 1 | 0 | 0 | 8 | 8 |
| | 4 | 1 | 3 | 4 | |
| | 16 | 5 | 2 | 1 | |
| *M. kansasii* | 1 | 15 | 4 | 1 | 20 |
| | 4 | 20 | 0 | 0 | |
| | 16 | 20 | 0 | 0 | |

(Continued on next page)

**TABLE 2** (Continued)

| Drug/species[a] | MIC (mg/liter) | *In vitro* DST category of isolates (*n*)[b] S | I | R | No. of isolates |
|---|---|---|---|---|---|
| Clofazimine | | | | | |
| *M. avium* | 0.25 | 1 | 4 | 75 | 80 |
| | 0.5 | 9 | 26 | 45 | |
| | 1 | 44 | 28 | 8 | |
| | 2 | 80 | 0 | 0 | |
| | 4 | 80 | 0 | 0 | |
| *M. chimaera* | 0.25 | 3 | 0 | 45 | 48 |
| | 0.5 | 24 | 5 | 19 | |
| | 1 | 39 | 8 | 1 | |
| | 2 | 48 | 0 | 0 | |
| | 4 | 48 | 0 | 0 | |
| *M. intracellulare* | 0.25 | 0 | 2 | 29 | 31 |
| | 0.5 | 2 | 12 | 17 | |
| | 1 | 17 | 12 | 2 | |
| | 2 | 30 | 1 | 0 | |
| | 4 | 31 | 0 | 0 | |
| Other MAC | 0.25 | 0 | 0 | 8 | 8 |
| | 0.5 | 1 | 1 | 6 | |
| | 1 | 4 | 2 | 2 | |
| | 2 | 8 | 0 | 0 | |
| | 4 | 8 | 0 | 0 | |
| *M. kansasii* | 0.25 | 9 | 3 | 8 | 20 |
| | 0.5 | 18 | 1 | 1 | |
| | 1 | 20 | 0 | 0 | |
| | 2 | 20 | 0 | 0 | |
| | 4 | 20 | 0 | 0 | |

[a]MAC, *M. avium* complex.
[b]The categories susceptible (S), intermediate (I), and resistant (R) are used in this study to describe presence or absence of *in vitro* growth at a defined drug concentration and neither represent clinical breakpoints nor predicted clinical outcome. Intermediate growth inhibition represents significant (>99%) but not complete inhibition and was categorized susceptible (S) for calculating MIC values and depicting distributions at the population level.

compared to members of the *M. avium* complex in our study. This is in line with findings that *M. kansasii* is in general more susceptible to NTM drugs than the *M. avium* complex and reports of a clofazimine ECOFF of 0.5 mg/liter for *M. kansasii* by Luo et al. (19).

Clofazimine resistance in NTM has been associated with mutations in the TetR family of regulators of adjacent MmpS5-MmpL5 efflux pumps: *mmpT5* in *M. intracellulare* (25) and MAB_2299 in *Mycobacterium abscessus* (26). The NTM isolates characterized in this study were therapy naive regarding clofazimine, and no elevated MICs were observed. Exploratory investigations into 10 randomly selected *M. chimaera* isolates did not reveal genetic diversity within the putative homologs RS13290 (*mmpt5*; 100% amino acid [aa] sequence identity) and RS24730 (MAB_2299; 70% aa sequence identity) of *M. chimaera* strain DSM 44623$^T$ (CP015278.1) (data not shown). In *M. tuberculosis*, mutations in the Rv0678 (*mmpR5*) locus are associated with clofazimine and bedaquiline resistance (27, 28). The closest homologs of Rv0678 were RS18640 (35% aa identity), RS15530 (35% aa identity), and RS06670 (24% aa identity) of *M. chimaera* DSM 44623$^T$ (data not shown). These findings confirm reports of others that there is no ortholog of Rv0678 (MmpR5) in the *M. avium* complex (25). Furthermore, unlike RS13290 and RS24730, the latter three genes are not located in the proximity of *mmpL* genes.

The MGIT 960/EpiCenter TB eXiST platform (Becton Dickinson) is recommended by the WHO for drug susceptibility testing *of M. tuberculosis*, including the testing of clofazimine, and therefore available in many mycobacteria laboratories worldwide (20). We have previously adapted MGIT 960 testing for automated quantitative drug susceptibility testing of slow-growing NTM and expanded this method for the testing of clofazimine within this study (12, 13). Commercial microdilution systems that lack clofazimine testing, e.g., the SLOWMYCO Sensititre panel from Trek Diagnostic Systems, are broadly used for drug

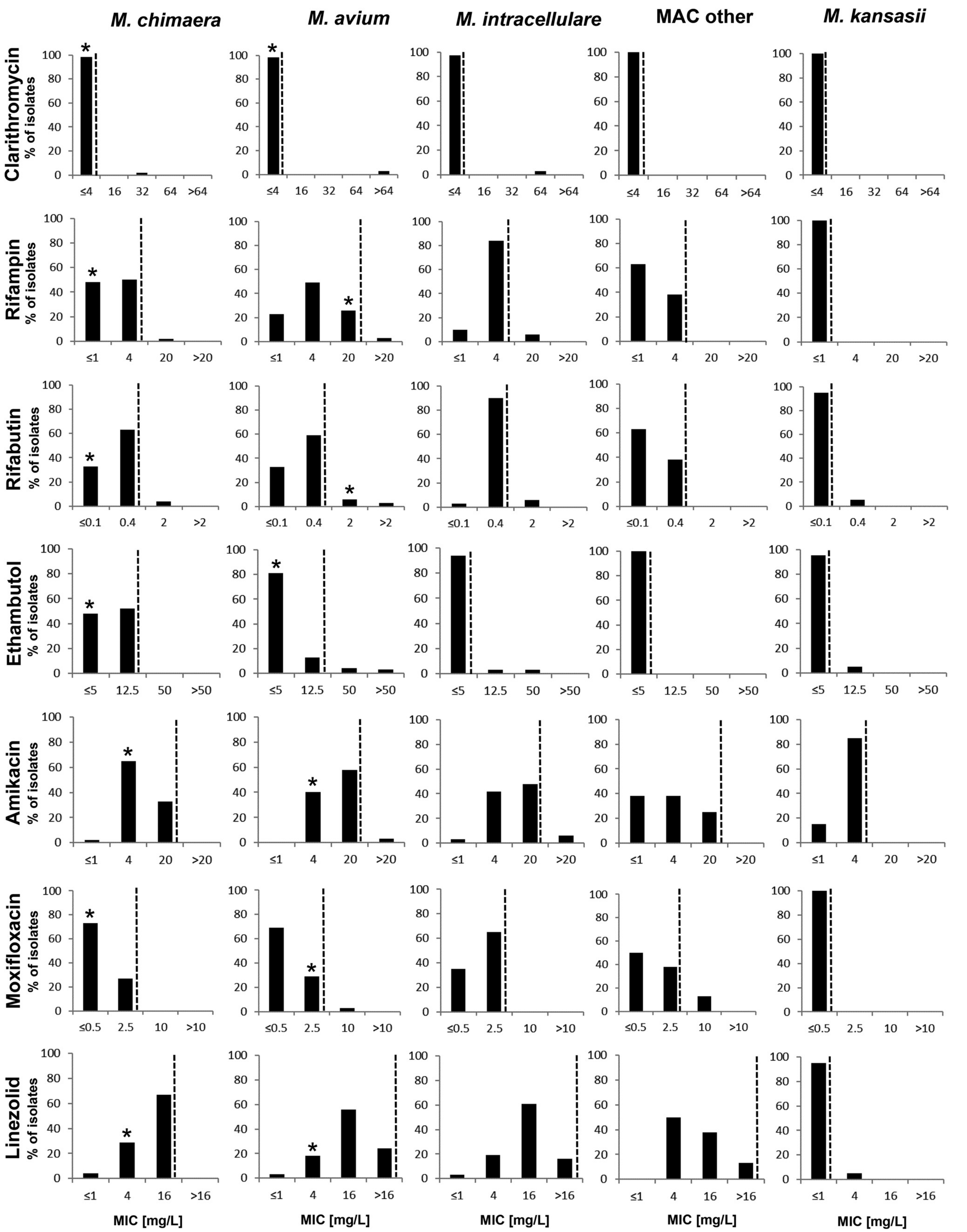


**FIG 2** Susceptibility distributions for different drugs and NTM species based on quantitative drug susceptibility testing data using MGIT TB eXiST. Approximated $MIC_{90}$ values are indicated (dashed line). MIC values of the type strains *M. avium* ATCC 19421 and *M. chimaera* DSM 44623 are indicated (*).

susceptibility testing of slow-growing NTM (8–11). MGIT 960 testing of clofazimine, a method established in many laboratories worldwide for *M. tuberculosis* complex, could complement commercial microdilution testing for slow-growing NTM in these laboratories. Our data support the addition of clofazimine to future commercial microdilution panels for NTM.

$MIC_{90}$ values of *M. chimaera* for drugs other than clofazimine, such as amikacin, clarithromycin, ethambutol, moxifloxacin, linezolid, and rifampin, are in agreement with the findings of previous studies for *M. chimaera* and comparable to values reported for the *M. avium* complex (1, 8–11, 22).

In conclusion, we provide MIC distribution, $MIC_{90}$, and ECOFF values of clofazimine for *M. chimaera* and demonstrate comparable values for other members of the *M. avium* complex. Further studies are needed to correlate *in vitro* susceptibility with clinical outcome.

## MATERIALS AND METHODS

**Mycobacterial strains and culture conditions.** Drug susceptibility was measured for 48 nonduplicate clinical isolates of *M. chimaera* and 139 additional slow-growing NTM from respiratory and nonrespiratory origin, including the *M. avium* complex isolates *M. avium* ($n=80$), *M. intracellulare* ($n=31$), *M. yongonense* ($n=3$), *M. timonense* ($n=2$), *M. bouchedurhonense* ($n=1$), *M. colombiense* ($n=1$), and *M. vulneris* ($n=1$), together with *M. kansasii* ($n=20$) isolates, that were submitted to or isolated at our mycobacteriological laboratory from 2014 to 2018 (Table 1). In addition, the type strains *M. avium* ATCC 19421 and *M. chimaera* DSM 44623 were analyzed. The isolates were identified by partial 16S rRNA gene sequence analysis as described previously (29). *M. kansasii* was differentiated by sequence analysis of the *hsp65* gene (30). Mycobacteria were grown in mycobacterium growth indicator tube (MGIT) medium supplemented with oleic acid albumin dextrose catalase (OADC) (Becton Dickinson, Sparks, MD) at 37°C.

**Drug susceptibility testing.** Drug susceptibility distributions of NTM were determined by automated, quantitative DST using the MGIT 960 system and the Epicenter TB eXIST system (Becton Dickinson) as previously described (12, 13). The antibiotics amikacin (1, 4, and 20 mg/liter), clarithromycin (4, 16, 32, and 64 mg/liter), clofazimine (0.25, 0.5, 1, 2, and 4 mg/liter), ethambutol (5, 12.5, and 50 mg/liter), linezolid (1, 4, and 16 mg/liter), moxiflocaxin (0.5, 2.5, and 10 mg/liter), rifabutin (0.1, 0.4, and 2 mg/liter), and rifampin (1, 4, and 20 mg/liter) were analyzed. Clofazimine was purchased from Sigma-Aldrich (Buchs, Switzerland) and dissolved in 100% dimethyl sulfoxide (DMSO). The terms susceptible (S), intermediate (I), and resistant (R) are used in this study to describe presence or absence of *in vitro* growth at a defined drug concentration and neither represent clinical breakpoints nor predicted clinical outcome. Intermediate growth inhibition represents significant (>99%) but not complete inhibition and was categorized susceptible (S) for calculating MIC values and depicting distributions at the population level.

**Clarithromycin and amikacin resistance analysis.** Phenotypic clarithromycin and amikacin resistance was confirmed by sequence analysis of the 23S rRNA gene and 16S rRNA gene, respectively, as described elsewhere (31, 32). Mutations at nucleotide positions A2058 and A2059 (*E. coli* equivalent) of the 23S rRNA gene were considered resistance markers for macrolides, and mutations at nucleotide position A1408 and C1409 (*E. coli* equivalent) of the 16S rRNA gene were considered amikacin resistance markers.

**Determination of ECOFF, $MIC_{50}$, and $MIC_{90}$ values.** MIC distributions were generated from the quantitative DST results. ECOFF values were determined by visual inspection of the MIC distributions (33). $MIC_{50}$ and $MIC_{90}$ were defined as drug concentrations that inhibit growth of 50% and 90%, respectively, of the population of a given species.

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