Short Communication

Minimum inhibitory concentration distributions for *Mycobacterium avium* complex—towards evidence-based susceptibility breakpoints

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**Article Info**

**Summary**

**Background:** Patients with clinical infections caused by the *Mycobacterium avium* complex (MAC) are treated for at least 1 year following sputum conversion with a regimen that suffers from a suboptimal cure rate. The correlation between clinical outcome and drug susceptibility testing breakpoints other than for the macrolides is regarded to be poor. A systematic evaluation of clinical breakpoints for MAC has not been performed so far; thus, the aim of this study was to initiate the process by establishing minimum inhibitory concentration (MIC) distributions.

**Methods:** The MICs of the major drugs used in the treatment of MAC infections were determined for 229 clinical MAC isolates in cation-adjusted Mueller–Hinton II broth.

**Results:** The MIC\textsubscript{50} and MIC ranges were established and compared to suggested susceptibility breakpoints for clarithromycin (2; 0.064–128 mg/l), rifabutin (0.25; <0.25–16 mg/l), ethambutol (8; 0.5–32 mg/l), amikacin (16; 1–128 mg/l), moxifloxacin (2; 0.25–16 mg/l), linezolid (32; 1–128 mg/l), rifampicin (8; 0.125–16 mg/l), and trimethoprim–sulfamethoxazole (2/38; 0.125/2–16/304 mg/l).

**Conclusions:** These results, together with those from available studies, indicate that MICs are high for drugs such as rifabutin, rifampicin, ethambutol, linezolid, and moxifloxacin used against MAC at levels unlikely to be associated with clinical efficacy at current dosing. This may partly explain the poor correlation between susceptibility testing and clinical outcomes for drugs other than clarithromycin.

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**Introduction**

The recommended duration of treatment for *Mycobacterium avium* complex (MAC) infections of 12 months following sputum conversion has a cure rate of around 75%, indicating a suboptimal strategy.\textsuperscript{1,2} Macrolides remain the cornerstone of treatment, together with a rifamycin and ethambutol.\textsuperscript{1} In severe cases, amikacin, clofazimine, or fluoroquinolones can also be considered.\textsuperscript{2,3} The correlation between the clinical outcomes of MAC infections and antimicrobial susceptibility testing (AST) breakpoints other than for the macrolides is regarded to be poor, although very limited supporting data exist.\textsuperscript{3} For MAC, the epidemiological cut-off point (ECOFF) and MIC distributions of wild-type isolates are not well described, and these are essential in establishing AST breakpoints for other bacteria together with clinical outcomes and pharmacokinetic/pharmacodynamic (PK/ PD) data.\textsuperscript{4} Thus, the aim of this study was to describe the wild-type MIC distributions of the drugs commonly used to treat MAC.

**Methods**

Following decontamination, specimens were inoculated into MGIT 960 tubes (BD, USA) and Löwenstein–Jensen slopes. Identification was performed by GenoType Mycobacterium CM test system (Hain Lifescience, Germany). A total of 229 MAC strains (157 *Mycobacterium avium*, 72 *Mycobacterium intracellulare* complex), isolated from 229 patients at the Karolinska University Laboratory between 2011 and 2015 were included. AST was performed by broth microdilution method using the SLOMYCO Sensititre panel (Trek Diagnostic Systems, USA). A 0.5–McFarland bacterial suspension was transferred to cation-adjusted Mueller–Hinton II broth (BD, USA) supplemented with 5% oleic albumin dextrose catalase (OADC). The MICs were read using the Sensititre Vizion System after an incubation period of 7–14 days at 37°C. The
MIC\textsubscript{50} was defined as the MIC required to inhibit the growth of 50% of the organisms. The reference strain \textit{M. avium} ATCC 700898 was included in each run as a quality control. The breakpoints for clarithromycin (S \leq 8 \text{ mg/l}), moxifloxacin (S \leq 1 \text{ mg/l}), and linezolid (S \leq 8 \text{ mg/l}) were those of the Clinical and Laboratory Standards Institute (CLSI).\textsuperscript{5}

**Results**

For clarithromycin, the MIC distribution of susceptible isolates ranged from 0.25 to 8 \text{ mg/l}, with a MIC\textsubscript{50} of 2 \text{ mg/l}. Six isolates showed resistance (MIC \geq 32 \text{ mg/l}) (Figure 1A).\textsuperscript{5} The MIC ranges for rifabutin and rifampicin were \leq 0.25–16 \text{ mg/l} and 0.125–16 \text{ mg/l}, respectively, with MIC\textsubscript{50} of \leq 0.25 and 8 \text{ mg/l}, respectively. The distribution for trimethoprim (TMP)–sulfamethoxazole (SMX) ranged from 0.125/2 \text{ mg/l} to 16/304 \text{ mg/l}, with a MIC\textsubscript{50} of 2/38 \text{ mg/l} (Table 1). Rifabutin was clearly truncated at the lower end of the MIC distribution (Figure 1B). For ethambutol the MIC\textsubscript{50} was 2 \text{ mg/l} and MIC range was 0.5–32 \text{ mg/l} (Figure 1C), and for amikacin MIC\textsubscript{50} was 16 \text{ mg/l} and the MIC range was 1–128 \text{ mg/l}. Applying the suggested PK/PD-derived breakpoints of van Ingen et al. (8 \text{ mg/l}),\textsuperscript{6} 93.5% of the isolates were susceptible to clarithromycin, whereas only 9.6% were susceptible to ethambutol (2 \text{ mg/l}) and 3% to rifampicin (0.5 \text{ mg/l}). For moxifloxacin, the MIC\textsubscript{50} was 2 \text{ mg/l} and the MIC range was 0.25–16 \text{ mg/l}; for linezolid, the MIC\textsubscript{50} was 16 \text{ mg/l} and the MIC range was 1–128 \text{ mg/l}.

![Figure 1](image-url)  
**Figure 1.** Minimum inhibitory concentration (MIC) distributions of drugs used against \textit{Mycobacterium avium} complex (MAC) infection: (A) clarithromycin (CLA); (B) rifabutin (RIB); (C) ethambutol (EMB). The pharmacokinetic/pharmacodynamic (PK/PD) breakpoints (Bp) as suggested by van Ingen et al., based on BACTEC 460 MIC determinations in combination with pharmacokinetic analyses, are included,\textsuperscript{7} as well as the Clinical and Laboratory Standards Institute (CLSI) suggested breakpoint for broth microdilution where applicable.\textsuperscript{5}
Table 1  
MIC distributions for alternative drugs used against Mycobacterium avium complex (MAC); the two-fold dilutions are given in mg/l.

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MIC, minimum inhibitory concentration; TMP, trimethoprim; SMX, sulfamethoxazole; PK/PD, pharmacokinetic/pharmacodynamic.

<sup>a</sup> Previously suggested breakpoints for MAC from PK/PD analyses.

<sup>b</sup> Clinical and Laboratory Standards Institute breakpoints for broth microdilution.

<sup>c</sup> The TMP concentration is 1/19 of the trimethoprim concentration (TMP = 1 mg/l, SMX = 19 mg/l).

(Table 1). Considering the tentative CLSI breakpoints for moxifloxacin (S ≤1 mg/l) and linezolid (S ≤8 mg/l), 15.3% and 15.7% of the isolates, respectively, would be susceptible. The quality control strain had a MIC variability of less than ±1 MIC dilution (n = 87) for all drugs tested and was within the CLSI target for clarithromycin.<sup>5</sup>

Discussion

In this study, MIC distributions were established for drugs used against MAC. It was found that for clarithromycin and TMP–SMX, the majority of strains had low MIC levels below the breakpoints for MAC and other mycobacteria.<sup>1,3,5,7–9</sup> Although no breakpoint has been established for amikacin, a similar rate of isolates below ≤16 mg/l (80.8%) was found, in agreement with a previous study (85.9%).<sup>7</sup> For the remaining drugs, and in particular for rifampicin, ethambutol, linezolid, and moxifloxacin, most isolates had MICs beyond the reach of therapeutic doses based on what is suggested by PK/PD, clinical outcome, and MIC distribution data for MAC,<sup>5,6,8</sup> even if synergy is taken into account. The evidence on which such drugs are recommended for the initial treatment of MAC is unclear and this has been based in part on clinical trials involving HIV patients in the pre-antiretroviral therapy era.<sup>1</sup>

It should be stressed that single MIC distributions should not be used to define clinical breakpoints for MAC. Rather, the present results illustrate the importance of such basic data to define clinical breakpoints along with PK/PD and clinical outcome studies.<sup>10</sup> However, for some drugs with generally very high MICs against MAC isolates, it is highly unlikely that clinical efficacy will be achieved, as this would imply that MAC behaves in a very unique way compared to other bacteria, including Mycobacterium tuberculosis, in the definition of clinical breakpoints.

This study has several limitations. Resistance genes were not sequenced and the treatment history of the patients was unknown. Additionally, there was a truncation in the rifabutin MIC distribution. It is clear that more MIC distributions from several laboratories need to be gathered before firm conclusions on ECOFFs can be drawn and clinical breakpoints defined together with PK/PD and clinical outcome data.

In summary, the MIC distributions for common drugs used against MAC have been presented, which indicate the potential clinical efficacy of clarithromycin and possibly sulfamethoxazole and amikacin.

Funding

This work was funded by the Swedish Heart and Lung Foundation (Oscar II Jubilee Foundation).

Conflict of interest

None to declare.

References