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Drug susceptibility distributions in slowly growing non-tuberculous mycobacteria using MGIT 960 TB eXiST

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ABSTRACT

In general, uniform clinical antibiotic susceptibility breakpoints (CBPs) for slowly growing nontuberculous mycobacteria (NTM) have not been established. The aim of this study was to determine wild-type drug susceptibility distributions for relevant antibiotics using Bactec MGIT 960 equipped with EpiCenter TB eXiST and to derive epidemiological cut-offs (ECOFFs) from semi quantitative drug susceptibility measurements. One hundred and twenty-six NTM clinical isolates (*Mycobacterium avium* $n = 58$, *Mycobacterium intracellulare* $n = 18$, *Mycobacterium kansasii* $n = 50$) were investigated in this study. Drug susceptibility distributions and MIC_{90} values were determined for clarithromycin, ethambutol, rifampicin, rifabutin, ofloxacin, moxifloxacin, and amikacin using Bactec MGIT 960/EpiCenter TB eXiST. For most species/drug combinations ECOFFs were determined. For some species/drug combinations ECOFFs were not defined as either the isolates were susceptible to the lowest drug concentration tested or because isolates, in part, had MIC levels exceeding the highest drug concentration tested. This study describes drug susceptibility distributions and MIC_{90} values of *M. avium*, *M. intracellulare*, and *M. kansasii* that may aid the definition of CBPs when correlating in vitro drug susceptibility with clinical outcomes in future studies.

Introduction

Diseases associated with slowly growing nontuberculous mycobacteria (NTM) are posing new challenges to clinicians and microbiologists since adequate antimycobacterial therapy is often problematic, or turns out to be unsuccessful (Griffith et al., 2007). The current guidelines of the British Thoracic Society (BTS) and the more recent guidelines of the American Thoracic Society (ATS) recommend clarithromycin based multi-drug regimens (Griffith et al., 2007; Subcommittee of the Joint Tuberculosis Committee of the British Thoracic Society, 1999). One of the most serious drawbacks of designing a potent therapeutic regimen for slowly growing NTM in the clinical practice is that current drug susceptibility testing of slowly growing NTM is lacking correlation with clinical outcome, with the exception of clarithromycin (Field et al., 2004; Griffith et al., 2007; Sison et al., 1996). Determination of drug susceptibility in slowly growing NTM can be method and species dependent (CLSI 2011). The Clinical and Laboratory Standards Institute (CLSI) provides method-dependent clinical breakpoints (CBPs) for clarithromycin in *Mycobacterium avium*. These CBPs are based on wild-type MIC distributions, studies of isolates with 23S rRNA mutations, and clinical studies. Uniform ECOFFs and CBPs for other drugs have not yet been established (CLSI 2011; Griffith et al., 2007; van Ingen et al., 2010, 2012a). For moxifloxacin and linezolid CLSI has proposed tentative breakpoints (CLSI 2011).

Determining ECOFFs using drug susceptibility distributions, comparing these ECOFFs with PK/PD data (if available), and subsequent selection of CBPs in clinical studies has been suggested as a reasonable strategy for setting CBPs (Turnidge and Paterson, 2007). However, this strategy warrants a reliable, adequately validated, and manageable routine system for quantitative drug susceptibility testing. In order to fulfil these requirements we have recently standardised and validated the fully automated Bactec Mycobacterium Growth Indicator Tube 960 system (MGIT 960/EpiCenter V5.53 system, equipped with the TB eXiST software module, Becton Dickinson, Franklin Lakes, NJ) for quantitative drug susceptibility testing of selected slowly growing NTM (Lucke et al., 2012; Springer et al., 2009). As a next step, the aim of this study was to determine wild-type distributions of quantitative levels of drug susceptibility, as these can be used to determine tentative ECOFFs. A total of 126 clinical slowly growing NTM isolates were included in this study representing all *M. avium*, *Mycobacterium intracellulare*, and *Mycobacterium kansasii* strains isolated over a 3-year-period in our diagnostic laboratory. These three species were chosen as the most frequently isolated clinically relevant species of slowly growing NTM (Brown-Elliott et al., 2012). The ECOFFs described in this study

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Table 1
Approximated MIC_{90} values for commonly used antibiotic drugs in NTM disease.

Drug/dose	Species	MIC_{90} (mg/L)	Drug concentrations tested (mg/L)
Clarithromycin	*M. avium*	≤4	4
	M. intracellulare	≤4	16
	M. kansasii	≤4	32
			64
Rifampicin	*M. avium*	>10	1
	M. intracellulare	≤10	4
	M. kansasii	≤1	10
Rifabutin	*M. avium*	≤2	0.1
	M. intracellulare	≤1	1
	M. kansasii	≤0.1	2
Ethambutol	*M. avium*	>25	5
	M. intracellulare	>25	25
	M. kansasii	25	50
Moxifloxacin	*M. avium*	≤2	0.5
	M. intracellulare	≤2	2
	M. kansasii	≤0.5	10
Ofloxacin	*M. avium*	>10	2
	M. intracellulare	>10	10
	M. kansasii	≤2	
Amikacin	*M. avium*	≤20	1
	M. intracellulare	≤10	4
	M. kansasii	≤10	10
			20

MIC_{90}s were derived from percentages of susceptibility at given concentrations (see Fig. 1 and Table 2).

may allow standardisation for drug susceptibility testing reports of slowly growing NTM, provide a meaningful aid on their interpretation to clinicians and pave the way towards further clinical studies to correlate drug susceptibility testing with clinical outcomes to develop CBPs.

Materials and methods

Bacterial strains

One hundred and twenty-six well-characterised slowly growing NTM isolates (*M. avium* $n = 58$, *M. intracellulare* $n = 18$, and *M. kansasii* $n = 49$) were investigated in this study for susceptibility to clarithromycin, rifampicin, rifabutin, ethambutol, ofloxacin, and moxifloxacin. For *M. avium*, *M. intracellulare*, and amikacin susceptibility data for more isolates were available from our records than for the other species/drug combinations ($n = 76$ and $n = 25$ for *M. avium* and *M. intracellulare*, respectively); the strains were isolated from patient specimens submitted to our diagnostic laboratory between 2009 and 2011. Mycobacterial isolates were identified by sequence analysis of the 16S rRNA gene as described previously (Rogall et al., 1990). Sequence analysis of *hsp65* was used to further identify *M. kansasii* (Telenti et al., 1993).

Antimicrobial agents

The antibiotics clarithromycin, rifampicin, rifabutin, ethambutol, ofloxacin, moxifloxacin, and amikacin (Sigma Aldrich Chemie GmbH, Buchs, Switzerland) were selected based on current diagnostic and treatment recommendations for *M. avium* complex (MAC) and *M. kansasii* (Brown-Elliott et al., 2012; Griffith et al., 2007; Kasperbauer and Daley, 2008). Drug concentrations tested are listed in Table 1.

Susceptibility testing using the MGIT 960 system with EpiCenter TB eXiST software

Drug susceptibility testing was performed as described previously (Lucke et al., 2012). In brief, positive MGIT tubes were subcultured for susceptibility testing as follows: (i) Subcultures were done within 1 or 2 days after the MGIT 960 system recorded a positive growth signal, bacterial suspensions with *M. intracellulare* and *M. kansasii* were diluted 1:5, and bacterial suspensions with *M. avium* were diluted 1:25 with sterile saline based on their different growth rate in the system. (ii) For subcultivations within 3–5 days after the MGIT 960 recorded a positive growth signal, bacterial suspensions with *M. intracellulare* and *M. kansasii* were diluted 1:25, and bacterial suspensions with *M. avium* were diluted 1:125 with sterile saline. The latter dilutions constituted the MGIT working suspensions. MGIT tubes supplemented with 0.8 mL of enrichment (BACTEC MGIT 960 SIRE Supplement; Becton Dickinson) were inoculated with 0.2 mL of the respective drug solutions and 0.5 mL of the working suspension. For the drug free growth control, the working suspension was diluted 1:100 with sterile saline solution (0.9%) and 0.5 mL of the diluted working suspension was inoculated (proportion method). The procedure for preparing the MGIT working suspensions was determined empirically to generate a positive signal with the drug free growth control within 4–10 days following inoculation.

MIC distributions and determination of ECOFFs

MIC distributions were determined using quantitative drug susceptibility testing data. Isolates with intermediate growth inhibition were included in the susceptible category (for details see Table 2). It is important to note that the terms susceptible (S), intermediate (I), and resistant (R) that we are applying to describe in vitro growth inhibition at a given drug concentration are not intended to be used to predict clinical outcome. The intermediate category indicates that the drug concentration examined significantly (>99%), but not completely, inhibits bacterial growth in vitro.

MIC_{90} approximations

MIC_{90} is defined as the drug concentration inhibiting the growth of 90% of mycobacterial isolates of a given species according to the 90th percentile in MIC distributions. MIC_{90}s were derived from percentages of susceptibility at given drug concentrations.

Results

Drug susceptibility distributions allowed the definition of tentative ECOFFs for most of the drugs examined (Fig. 1, for detailed data see Table 2).

Clarithromycin

For *M. avium* the tentative ECOFF was defined as ≤4 mg/L (Fig. 1). Distinct ECOFFs for *M. kansasii* and *M. intracellulare* could not be proposed since all isolates were susceptible at the lowest concentration tested (ECOFF ≤4 mg/L). One *M. avium* isolate showed a disproportionally high MIC of >64 mg/L, however this isolate had an A2059C point mutation in the 23S rRNA (Meier et al., 1994).

Rifampicin and rifabutin

A tentative rifampicin ECOFF could not be defined for *M. avium*, as a significant proportion of isolates (15.5%) had MICs exceeding the highest drug concentrations tested (10 mg/L, Fig. 1). The inferred rifampicin tentative ECOFF for *M. intracellulare* was 4 mg/L.

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