Significant Difference in Drug Susceptibility Distribution between *Mycobacterium avium* and *Mycobacterium intracellulare*

Aurélien Renvoisé,^{a,b,c} Christine Bernard,^{a,b,c} Nicolas Veziris,^{a,b,c} Eve Galati,^{a*} Vincent Jarlier,^{a,b,c} Jérôme Robert^{a,b,c}

AP-HP, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Bactériologie-Hygiène, Paris, France^a; Sorbonne Universités, UPMC Univ Paris 06, U1135, Center for Immunology and Microbial Infections, Team 13, Paris, France^b; INSERM, U1135, Center for Immunology and Microbial Infections, Team 13, Paris, France^c

Mycobacterium avium and *M. intracellulare* are referred as the *M. avium* complex (MAC) (1) and are known as causative agents of opportunistic infections in humans (1–3). The first-line recommended treatment consists of a combination of either clarithromycin or azithromycin with rifampin and ethambutol, the

two latter drugs being considered companion drugs to prevent the emergence of macrolide resistance (4, 5). Amikacin is an alternative to macrolides for the treatment of MAC lung cavity disease or in case of macrolide resistance (6). To date, the treatment guidelines have referred to MAC as a complex (4), but there are no clear data regarding the antibiotic susceptibility of each species separately. Therefore, we sought to delineate the clarithromycin and amikacin susceptibility profiles of both species (4, 5).

All of the clinically relevant MAC isolates sent to the French National Reference Center for Mycobacteria between 2009 and 2011 were routinely identified by using GenoType Mycobacterium CM/AS line probe assays (Hain, Lifescience) as recommended by the manufacturer, leading to the identification of *M. avium* or *M. intracellulare* (7). After the exclusion of duplicates, only isolates from patients with no history of previous antibiotic treatment were selected to be representative of a wild population. MICs were determined by the broth microdilution method with Sensititre SLOMYCO (Biocentric) (8). The Kruskal-Wallis test was used for statistical comparisons. Epidemiological cutoff (ECOFF) values were determined as values larger than the modal MIC plus one 2-fold dilution (variability of the test) and including at least 95% of the isolates tested.

One hundred eighty-six *M. avium* and 154 *M. intracellulare* isolates were studied. The distributions of clarithromycin and amikacin MICs were unimodal for both species (Fig. 1). Clarithromycin MICs were lower for *M. intracellulare* than for *M. avium* isolates (MIC₅₀, 2 versus 8 mg/liter, respectively; $P < 0.001$) (Table 1). The same observation was made for amikacin MICs, although the difference was not as drastic (MIC₅₀, 8 versus 16 mg/liter, respectively; $P < 0.001$). Consequently, the tentative ECOFFs were slightly lower for *M. intracellulare* than for *M. avium* (Table 1).

Many studies report MAC as a single entity. Our results demonstrate that *M. avium* and *M. intracellulare* have different patterns of clarithromycin and amikacin susceptibility. Such a difference has been previously reported (9, 10), but recent data considering both clarithromycin and standardized methods (recommended by CLSI [8]) were lacking at the time of those publi-

FIG 1 Distribution of clarithromycin (a) and amikacin (b) MICs for *M. avium* and *M. intracellulare* and box plots of MIC distribution (c).

Published ahead of print 1 October 2014

Editor: M. J. Loeffelholz

Address correspondence to Jérôme Robert, jerome.robert0@upmc.fr.

* Present address: Eve Galati, Laboratoire AMP, Center Hospitalier de Dreux, Dreux, France.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.02127-14

TABLE 1 Statistical analysis of MICs of clarithromycin and amikacin for MAC

Drug and species no. of isolates)	Modal MIC	MIC ₅₀	MIC ₉₀	Geometric mean MIC	MIC range	ECOFF
Clarithromycin						
<i>M. avium</i> (186)	8 ^a	8	16	6.7	1–16	16
<i>M. intracellulare</i> (154)	2	2	4	1.7	≤0.25–16	8
Amikacin						
<i>M. avium</i> (186)	16	16	16	10.7	2–32	32
<i>M. intracellulare</i> (154)	8	8	16	7.4	2–32	16

^a Values are in milligrams per liter.

cations. Considering that (i) clarithromycin and amikacin MICs have been linked to treatment outcomes (6, 11) and (ii) the MICs for *M. avium* are higher, one could expect more therapeutic failures and a higher rate of selection of resistant mutants of this species if it is associated with a large mutant selection window (which remains to be determined). A limitation of the present study is, however, the use the GenoType Mycobacterium CM/AS line probe assay to identify *M. avium* and *M. intracellulare*. Other MAC species, such as *M. chimaera*, may have been misidentified as one of the two main species by this assay, although the impact on the results is likely to be limited because the other species are less frequently encountered (7, 12). In fine, our results need to be further confirmed by a definitive species identification method, such as sequencing.

In conclusion, there are differences in antibiotic susceptibility between MAC species in terms of MIC₅₀s and ECOFFs. Whether these differences are clinically relevant remains to be determined; indeed, there are not a wide variety of drugs and dosage options for the treatment of MAC infections, and the choice of the antibiotic regimen could be based on MICs determination despite definite species identification. On the other hand, these differences will be of interest in determining the correlation between *in vitro* results and clinical and bacteriological outcomes.

ACKNOWLEDGMENTS

We are grateful to Stanley Pang for editing the manuscript and to Anton Granzhan for his help in preparing the manuscript.

We have no conflict of interest to declare.

This work was supported by an annual grant from the Institut Na-

tional de Veille Sanitaire to the French National Reference Center for Mycobacteria and Antimycobacterial Resistance.

REFERENCES

- Esteban J, Garcia-Pedrazuela M, Munoz-Egea MC, Alcaide F. 2012. Current treatment of nontuberculous mycobacteriosis: an update. *Expert Opin. Pharmacother* 13:967–986. <http://dx.doi.org/10.1517/14656566.2012.677824>.
- van Ingen J. 2013. Diagnosis of nontuberculous mycobacterial infections. *Semin. Respir. Crit. Care Med.* 34:103–109. <http://dx.doi.org/10.1055/s-0033-1333569>.
- Dailloux M, Abalain ML, Laurain C, Lebrun L, Loos-Ayav C, Lozniewski A, Maugein J. 2006. Respiratory infections associated with nontuberculous mycobacteria in non-HIV patients. *Eur. Respir. J.* 28:1211–1215. <http://dx.doi.org/10.1183/09031936.00063806>.
- Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Jr, Winthrop K. 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am. J. Respir. Crit. Care Med.* 175:367–416. <http://dx.doi.org/10.1164/rccm.200604-571ST>.
- Brown-Elliott BA, Nash KA, Wallace RJ, Jr. 2012. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. *Clin. Microbiol. Rev.* 25:545–582. <http://dx.doi.org/10.1128/CMR.05030-11>.
- Brown-Elliott BA, Iakhiaeva E, Griffith DE, Woods GL, Stout JE, Wolfe CR, Turenne CY, Wallace RJ, Jr. 2013. *In vitro* activity of amikacin against isolates of *Mycobacterium avium* complex with proposed MIC breakpoints and finding of a 16S rRNA gene mutation in treated isolates. *J. Clin. Microbiol.* 51:3389–3394. <http://dx.doi.org/10.1128/JCM.01612-13>.
- Russo C, Tortoli E, Menichella D. 2006. Evaluation of the new GenoType Mycobacterium assay for identification of mycobacterial species. *J. Clin. Microbiol.* 44:334–339. <http://dx.doi.org/10.1128/JCM.44.2.334-339.2006>.
- Clinical and Laboratory Standards Institute. 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard, 2nd ed. Clinical and Laboratory Standards Institute, Wayne, PA.
- Saito H, Tomioka H, Sato K, Tasaka H, Tsukamura M, Kuze F, Asano K. 1989. Identification and partial characterization of *Mycobacterium avium* and *Mycobacterium intracellulare* by using DNA probes. *J. Clin. Microbiol.* 27:994–997.
- Hombach M, Somoskovi A, Homke R, Ritter C, Bottger EC. 2013. Drug susceptibility distributions in slowly growing non-tuberculous mycobacteria using MGIT 960 TB eXiST. *Int. J. Med. Microbiol.* 303:270–276. <http://dx.doi.org/10.1016/j.ijmm.2013.04.003>.
- Kobashi Y, Abe M, Mouri K, Obase Y, Kato S, Oka M. 2012. Relationship between clinical efficacy for pulmonary MAC and drug-sensitivity test for isolated MAC in a recent 6-year period. *J. Infect. Chemother.* 18:436–443. <http://dx.doi.org/10.1007/s10156-011-0351-x>.
- Tortoli E, Pecorari M, Fabio G, Messino M, Fabio A. 2010. Commercial DNA probes for mycobacteria incorrectly identify a number of less frequently encountered species. *J. Clin. Microbiol.* 48:307–310. <http://dx.doi.org/10.1128/JCM.01536-09>.