

Fanourios Kontos¹, Marina Zande², Julie Catherine Herrera Diaz², Dimitrios Mavromanolakis², Zoe Gitti^{2*}.

¹ Clinical Microbiology Laboratory, Medical School of Athens, Attikon University Hospital, Athens, Greece, e-mail: fankon68@gmail.com.

² Laboratory of Clinical Microbiology, University Hospital of Heraklion, Crete, Greece, * zgitti@gmail.com.

Objectives. *Mycobacterium avium* complex (MAC) including a group of mycobacterial pathogens, composed of 10 species [1], most commonly isolated from respiratory samples. In addition, MAC infection can also result in lymphadenitis and disseminated disease in both immunocompromised and immunocompetent patients [2]. Clinical indications of performing susceptibility testing are predefined while clarithromycin is considered as the drug of choice [2]. The aim of our study is to investigate the clinical significance and the susceptibilities of MAC isolates recovered from clinical specimens in Crete, Greece.

Methods. We studied 42 MAC isolates recovered from different patients during the decade 1/2001-12/2010. For identification, the GenoType Common Mycobacteria (CM) (Hain Lifescience, Nehren, Germany) and sequencing analysis of hsp65 (440bp) and rpoB (711bp) genes were performed. [3,4]. For the determination of the clinical relevance of MAC isolates, the established bacteriological criteria for NTM lung disease by the American Thoracic Society (ATS) were used [5].

The minimum inhibitory concentration (MIC) in µg/ml was determined with the standard broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) [6] using a commercial assay (SLOMYCOI, TREK Diagnostic systems). Isolates were tested against the primary drug clarithromycin (CLA), the secondary drugs moxifloxacin (MXF) and linezolid (LIN) and the clinically useful drugs streptomycin (STR), ethambutol (EMB), rifampicin (RIF), rifabutin (RFB) and amikacin (AMK). The breakpoints of resistance to CLA, and the tentative breakpoints to LIN and to MXF were recommended by CLSI [6] (Table 1) and breakpoints of resistance to STR, EMB, RIF, and AMK, were recommended by previous literatures [7,8] (Table1). Finally the MIC₅₀ and MIC₉₀ for all antibiotics tested were determined.

Antimicrobial Agent	Minimal Inhibitory Concentration (µg/ml) for category (breakpoints)			Range of Concentrations (µg/ml) [Number of Concentrations]
	S	I	R	
CLA	≤8	16	≥32	0.06-64 [11]
MXF	≤1	2	≥4	0.12-8 [7]
LIN	≤8	16	≥32	1-64 [7]
EMB			≥8	0.5- 16 [6]
RIF			≥8	0.12-8 [7]
AMI			≥32	1-64 [7]
STR			≥8	0.5-64 [8]
RFB			≥1	0.25-8 [6]

Table 1. Broth microdilution interpretive criteria for MAC as suggested by the literature (6,7,8), range and number of the respective antibiotic concentrations tested by the microdilution panel SLOMYCOI. S: susceptible, I:Intermediate, R:Resistant

Identification	Specimen type	No of strains	No of clinically significant strains
Respiratory specimens (n= 35)			
<i>M.avium</i>	Sputum	22	14
<i>M.avium</i>	Gastric fluid	3	2
<i>M.avium</i>	BAL	1	1
<i>M. marseillense</i>	Sputum	3	3
<i>M. marseillense</i>	BAL	2	2
<i>M. chimaera</i>	Sputum	2	
<i>M. intracellulare</i>	Sputum	1	
<i>M. timonense</i>	Sputum	1	
Non-respiratory specimens (n=7)			
<i>M.avium</i>	Lymph node biopsy	4	4
<i>M.avium</i>	Blood	3	3

Table 2. Distribution of MAC species isolated from clinical specimens.

✓Five MAC species were recovered: *M.avium* (n=33), *M.marseillense*(n=5), *M.chimaera* (n=2), *M.timonense* (n=1) and *M.intracellulare* (n=1).
 ✓Between 42 MAC strains 35 (83%) isolated from pulmonary specimens and the 63% (22/35) considered as clinically significant according to the ATS criteria.
 ✓There were three cases of disseminated *M.avium* disease in HIV(+) patients. Another one *M.avium* strain recovered from a sputum specimen of a HIV(+) patient.

Results

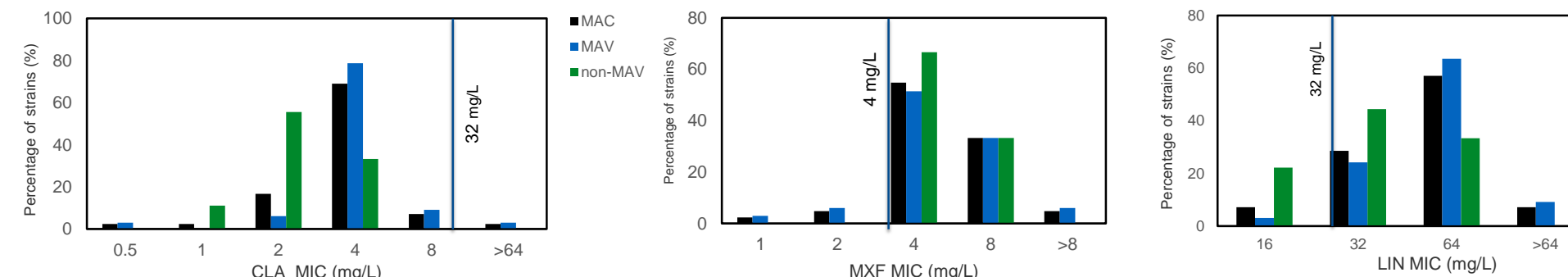


Fig1. Distribution of CLA, MXF, and LIN, MICs for MAC, M. avium (MAV) and non-MAV isolates. Blue lines represents the resistance breakpoints.

Agent	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	range (µg/ml)	Number of susceptible (%)/ resistant strains (%)	
				MAC	MAV
CLA	4	8	0.5->64	41(97.6)/ 1(2.4)	32(97)/ 1(3)
LIN	64	64	16->64	0/ 39(93)	0/ 32(97)
MXF	4	8	1- >8	3(7.1)/ 37(88)	1(3)/ 30(91)
AMK	32	32	8-64	13(31)/ 29(69)	10(30.3)/ 23(69.7)
STR	64	>64	MAC: 8- >64 MAV: 16->64	0/ 42(100)	0/ 33(100)
EMB	8	16	4->16	2(4.8)/ 40(95.2)	2(6)/ 31(96)
RIF	>8	>8	2->8	15(36)/ 27(64)	13(39)/ 21(61)
RFB	0.5	2	<0.25-4	23(55)/ 19(45)	18(55)/ 15(45)

Table 3. Susceptibilities of the 42 MAC and 33 M.avium (MAV) strains to 8 antimicrobial agents.

Strain No	Species	(MIC µg/ml) (S: Susceptible, R:Resistant, Intermediate)							
		CLA	LIN	MXF	AMK	RIF	STR	EMB	RFB
1	<i>M. marseillense</i>	2(S)	16(I)	4(R)	16(S)	4(S)	64(R)	8(R)	2(R)
2	<i>M. marseillense</i>	2(S)	32(R)	4(R)	32(R)	8(R)	64(R)	8(R)	4(R)
3	<i>M. marseillense</i>	2(S)	64(R)	8(R)	32(R)	>8(R)	>64(R)	8(R)	0.5(S)
4	<i>M. marseillense</i>	4(S)	32(R)	4(R)	32(R)	8(R)	64(R)	8(R)	0.5(S)
5	<i>M. marseillense</i>	4(S)	64(R)	8(R)	64(R)	8(R)	>64(R)	>16(R)	1(R)
6	<i>M. chimaera</i>	2(S)	32(R)	4(R)	16(S)	2(S)	64(R)	8(R)	<0.25(S)
7	<i>M. chimaera</i>	4(S)	64(R)	8(R)	32(R)	4(S)	>64(R)	8(R)	<0.25(S)
8	<i>M. intracellulare</i>	2(S)	32(R)	4(R)	32(R)	8(R)	64(R)	8(R)	4(R)
9	<i>M. timonense</i>	1(S)	16(I)	4(R)	16(S)	8(R)	8(R)	8(R)	0.5(S)

Table 4. Susceptibilities of the 9 MAC "non-M. avium" species to 8 antimicrobial agents.

REFERENCES

- Tortoli E. Clin. Microbiol. Rev. 2014; 27(4):727.
- Brown-Elliott B., Nash K.A., Wallace R.J.J 2012. Clin. Microbiol. Rev. 2014; 25(3): 545-582.
- Ben Salah I., et al. Microbiology 2008; 154, 3715-3723.
- Telenti, et al. J Clin Microbiol 1993; 31: 175-78.
- Griffith et al 2007, Am J Respir Crit Care Med, 2007; 175: 367-416.
- Clinical and Laboratory Standards Institute (CLSI), 2011, Document M24-A2, Vol. 31, No 5.
- Heifets L. Antimicrob. Agents Chemother. 1988; 32: 1131-1136.
- Brown-Elliott B. et al. J Clin Microbiol 2013; 51: 3389-3394.

✓The >97% of MAC strains were susceptible to clarithromycin. Only one strain was resistant to clarithromycin. This is a *M.avium* strain which was recovered from a blood specimen of a HIV(+) patient with a MIC of >64 µg/ml.
 ✓On the contrary the percentages of MAC strains resistant to the secondary drugs linezolid and moxifloxacin is >85%.
 ✓For the other drugs the percentages of resistant strains is between 45 to 100%
 ✓There were no differences in susceptibilities between *M.avium* and the other "non-*M.avium*" stains.

Conclusion.

✓ This study indicates the distribution of species, the clinical relevance and the existing sensitivity/ resistance of MAC isolates recovered in Crete, Greece.
 ✓The drug of choice clarithromycin revealed highly antimicrobial activity against MAC isolates
 ✓Moxifloxacin, linezolid and the other drugs tested showed poor activity *in vitro*.
 ✓The correlation between *in vitro* activity and the outcome of treatment for MXF, LIN, AMK STR, RIF, RFB and EMB will validate this conclusion and establish the correlation of *in vitro* results with clinical response.