In vitro antimicrobial susceptibility of clinical and environmental *Mycobacterium chimaera* isolates

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Background

Mycobacterium chimaera is a slow-growing nontuberculous *Mycobacterium* sp. belonging to the *Mycobacterium avium* Complex (MAC). It has been identified globally as the cause of a large outbreak of cardiac infections following open heart surgery, and can cause respiratory infections in individuals with underlying structural lung disease. The optimal antibiotic treatment regimen for these infections is unknown. In this study, the *in vitro* susceptibility of *M. chimaera* was investigated for antimicrobial agents commonly considered for use to treat MAC infections.

Aims

To determine the susceptibility profile of clinical and environmental *M. chimaera* isolates to antimicrobial agents (clarithromycin, moxifloxacin, linezolid, rifampicin, rifabutin, ethambutol and amikacin) that are commonly considered for use in the treatment of MAC infection.

Materials and Methods

***M. chimaera isolates*:** A total of 87 *M. chimaera* isolates (61 of respiratory origin, 15 associated with cardiac surgery, and 11 isolated from water samples taken from heater-cooler units) were studied. All isolates were identified as *M. chimaera* by 16S rRNA and ITS gene sequencing and confirmed using the GenoType NTM-DR assay (HAIN Lifescience). *The M. chimaera* DSM44623 reference strain was included (1).

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using the SLOWMYCO Sensititre® panel (TREK Diagnostic Systems). The MIC was determined for clarithromycin, rifampicin, rifabutin, moxifloxacin, ethambutol, linezolid and amikacin for all *M. chimaera* isolates and for a *M. chimaera* DSM44623 reference strain. CLSI breakpoints were available for clarithromycin and tentative breakpoints were available for moxifloxacin and linezolid only(2). Proposed breakpoints from the literature were used to interpret strains as being susceptible, intermediate and resistant to rifampicin, amikacin and ethambutol (3,4).

Results

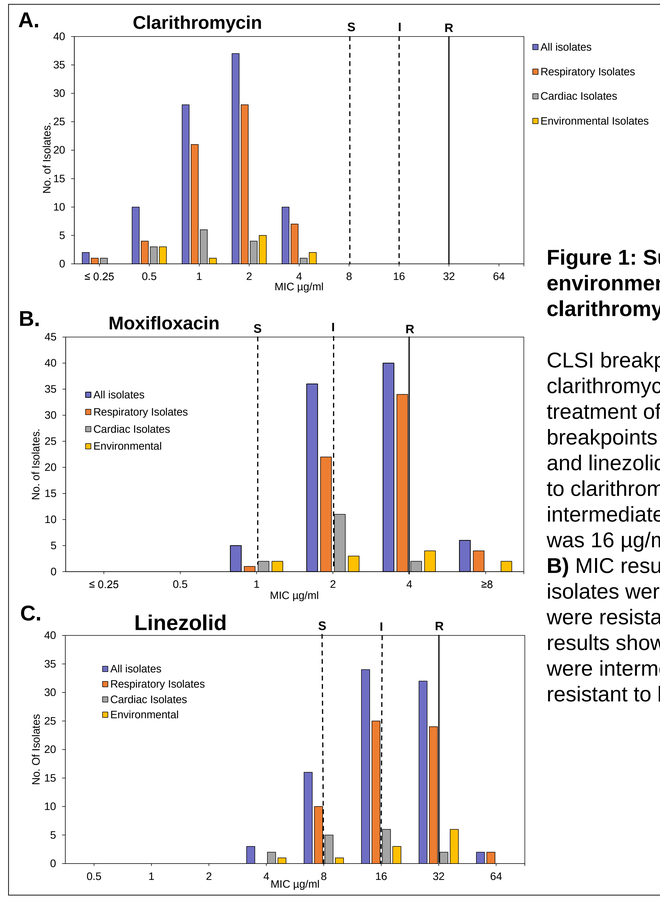

Figure 1: Susceptibility of clinical and environmental *M. chimaera* isolates to clarithromycin, moxifloxacin and linezolid

CLSI breakpoints were available for clarithromycin as the primary drug for treatment of MAC infections and tentative breakpoints were available for moxifloxacin and linezolid. **A)** All isolates were susceptible to clarithromycin (<8 µg/ml) and the intermediate (I) and resistant breakpoint (R) was 16 µg/ml and 32 µg/ml respectively. **B)** MIC results show that 41% (36/87) of isolates were intermediate and 53% (46/87) were resistant to moxifloxacin. **C)** MIC results show that 39% (34/87) of isolates were intermediate and 39% (34/87) were resistant to linezolid.

Table 1: The antimicrobial susceptibility pattern of clinical and environmental *M. chimaera* isolates

Isolate Source	Antimicrobial Agent*	MIC (µg/ml)									% of Resistant strains**
		≤ 0.25	0.5	1	2	4	8	16	32	64	
Respiratory (n= 61)											
	CLA	1	4	21	28	7					0
	MOXI			1	22	34	4				61
	LIN						10	25	24	2	42
	RIF			5	21	27	8				13
	EMB				11	41	6	3			15
	RFB	27	23	9	1	1					0
	AMI				1	4	29	11			0
Cardiac (n=15)											
	CLA	1	3	6	4	1					0
	MOXI			2	11	2					13
	LIN					2	5	6	2		13
	RIF			3	5	6	1				7
	EMB		2		4	8	1				7
	RFB	11	3	1							0
	AMI				1	6	6	1	1		7
Environmental (n=11)											
	CLA		3	1	5	2					0
	MOXI			2	3	4	2				55
	LIN					1	1	3	6		55
	RIF		1		1	2	7				64
	EMB		0		3	8					0
	RFB	7	2	1			1				0
	AMI					3	5	2	1		9

*Antimicrobial agents represent clarithromycin (CLA), moxifloxacin (MOX), linezolid (LIN) , rifampicin (RIF), ethambutol (EMB), rifabutin (RFB) and amikacin (AMI) ** CLSI Breakpoints were used to interpret susceptible, intermediate and resistant MIC's to the following antimicrobial agents: Clarithromycin S ≤ 8 µg/ml , I=16 µg/ml, R ⩾ 32 µg/ml , Moxifloxacin S ≤ 1 µg/ml , I = 2 µg/ml, R ⩾ 4 µg/ml , and Linezolid S ≤ 8 µg/ml, I = 16 µg/ml, R ⩾ 32 µg/ml. *M. chimaera* isolates were considered to be resistant if the MIC for Rifampicin ⩾ 8 µg/ml , EMB ⩾ 8 µg/ml and AMI ⩾ 32µg/ml, as proposed by Kim et al 2013 and Brown-Elliot et al 2013 (3,4).

Conclusions

- Clarithromycin is the primary drug for treatment of MAC infection and all *M. chimaera* isolates were susceptible which indicated very good *in vitro* activity against *M. chimaera.*
- Ethambutol, rifampicin, rifabutin and amikacin are considered clinically useful agents for treatment of MAC infection and these antimicrobial agents had good *in vitro* activity against most clinical *M. chimaera* isolates in our study.
- There was a high prevalence of non-susceptible isolates to moxifloxacin and linezolid in our study.
- *M. chimaera* DSM44623 strain was susceptible to clarithromycin, linezolid, rifampicin, rifabutin, ethambutol and amikacin, but was intermediate to moxfloxacin (data not shown).
- The approach to treatment of *M. chimaera* infections is determined by more than just *in vitro* antimicrobial susceptibility but our results should help inform on the choice of antimicrobial agents as part of the overall therapeutic strategy.

Future Work

- Whole genome sequencing of *M. chimaera* isolates will be used to explore the diversity of strains associated with antimicrobial resistance or reduced susceptibility to certain antimicrobial agents.
- Drug resistance gene targets (*rpoB, embB, rrs, gyrA, gyrB*, 23S rRNA gene) for rifampicin, ethambutol, amikacin, moxifloxacin and linezolid will be investigated for acquired chromosomal mutations.

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