O088
Oral Session
Deep insights in mycobacteria: genomics and susceptibility

PHENOTYPIC AND GENETIC CHARACTERIZATION OF AMINOGLYCOSIDES AND MACROLIDES RESISTANCE IN CLINICAL ISOLATES OF MYCOBACTERIUM ABSCESSUS

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Objectives:
To study resistance to aminoglycosides and macrolides for clinical isolates of *Mycobacterium abscessus*.
Methods:
50 *M. abscessus* clinical isolates were studied for MICs of amikacin, tobramycin and clarithromycin using the broth microdilution, Sensititre RAPMYCO plates (Trek Diagnosis Systems, Biocentric, France). To differentiate clarithromycin inducible resistance from susceptible isolates, plates were submitted to an extended incubation as previously described (Bastian et al. 2011), with successive readings after 5, 7, and 14 days of incubation at 30°C. In addition *rrl* (23s ribosomal RNA), *rrs* (16s ribosomal RNA) and *erm41* (methyl transferase) genes w ere sequenced. The isolates were identified using *hsp65* gene sequencing.
Results:
For the 44 *M. abscessus* isolates that did not show *rrs* gene mutations, the amikacin MIC w as ≤ 16 mg/l for 30 (60%) isolates, 32 mg/l for 9 isolates and ≥ 64 mg/l for 5 isolates. *rrs* was not determined for 2 isolates: one with amikacin MIC value ≤ 16 mg/l, the other with MIC value ≥ 64 mg/l. For the 4 isolates harbouring a A1408G *rrs* mutation, known to confer amikacin resistance, MICs were ≥ 64 mg/l.
Regarding to the clarithromycin, there was a relation between the *erm41* gene and the intrinsic resistance: 29 *M. abscessus abscessus* with an *erm41 sequevar T28* and 5 *M. bolletii* show ed inducible resistance to clarithromycin; *5 M. abscessus* with an *erm41 sequevar* C28 and 3 *M. massiliense* were susceptible to clarithromycin w ith MIC below 1 mg/l. *erm41* was not determined for 3 isolates among whom one w as susceptible to clarithromycin and the 2 others showed inducible resistance. In addition, we observed a high level of constitutive resistance to clarithromycin for 5 isolates with MICs > 16 mg/l out of w hich 4 isolates harboured a A2058G *rrl* mutation.

Conclusions:
Whereas intrinsic resistance and sensitivity to clarithromycin w as associated to the *erm41* genotype in *M. abscessus*, a high level resistance to clarithromycin (found in 10% of *M. abscessus* clinical isolates) w as associated to *rrl* mutations in 3 isolates out of 4. As previously described (Bastian et al. 2011), genotypic pattern including *rrl* and *erm41* genes is correlated with clarithromycin susceptibility. For amikacin, the MIC value was less correlated w ith the detection of *rrs* mutations probably because of the narrow range of concentration values provided in the commercially available microdilution testing. High resistance to amikacin w ithout *rrs* mutation in *M. abscessus* may be due to other mutation in ribosomal protein (*rps*) or aminoglycoside 2-N-acetyltransferase, w hich are under investigation.
Reference(s):
Bastian, S., et al., Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm(41)* and *rrl* sequencing. Antimicrobial agents and chemotherapy, 2011. 55(2): p. 775-81.

Figure1. amikacin and clarithromycin MICs in 50 *M. abscessus* clinical isolates (broth microdilution, Sensititre RAPMYCO plates)

Antibiotic MICs (mg/L)(nb of isolates)	***rrs* gene sequences** (*E. coli* numbering)			
	WT	A1408G mutation	other *rrs* mutations	ND
Amikacin				
≤ 16	30			1
32	9			
≥ 64	5	4		1
	***rrl* gene sequences** (*E. coli* numbering)			
	WT	2058-2059 mutations	other mutations	ND
Clarithromycin				
≤ 2	9			
≥ 16	34* + 1	3 (A2058G)	2* (G2068A)	1*

*inducible resistance after prolonged incubation (14 days)
ND: not determined