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Mutations in 23S rRNA among *Pseudomonas aeruginosa* isolates from Cystic Fibrosis patients confer higher resistance to macrolides.

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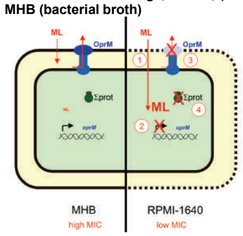
Background

Cystic fibrosis (CF) is an autosomal recessive genetic disease, affecting mainly Caucasian population. It is characterized by overproduction of sticky, thick mucus in many organs such as the lung, pancreas and gastrointestinal tract. This offers an ideal environment for proliferation of opportunistic pathogens.

P.aeruginosa is the main microorganism causing chronic respiratory tract infections in CF patients older than 25 years (1). These patients, therefore, require repetitive and prolonged antibiotic treatments with anti-pseudomonal drugs.

The majority of these patients also receive long-term macrolide treatment for their anti-inflammatory properties. This could possibly give rise to mutations on macrolide's target (domain V of 23S subunit of rRNA) in *P.aeruginosa* (2). On the other hand, patients suffering from *P.aeruginosa* healthcare-associated pneumonia characterized by acute respiratory infection do not receive macrolide treatment.

Figure 1: Mechanism of action of macrolides in biological media (RPMI [culture medium for eukaryotic cells]; broncho-alveolar lavage, serum,) vs MHB (bacterial broth)



In CA-MHB macrolides show a high MIC because of a poor diffusibility through the outer membrane and active efflux by the constitutively-expressed efflux systems MexAB-OprM and MexXY-OprM. In RPMI-1640 medium, the outer membrane becomes more permeable, which favors macrolide penetration within bacteria (1), allows them to impair, through a still undefined mechanism, the expression of *oprM* (2), and therefore reduces the activity of the efflux systems MexAB-OprM and MexXY-OprM (3). In combination, steps 1 and 4 allows for an increased intracellular accumulation of macrolides, and allows them to impair protein synthesis at lower extrabacterial concentrations (4) and, thereby, to exert true antibacterial activity.

Macrolides are deemed to be ineffective against *P.aeruginosa*, showing high MICs in conventional broth. However, our laboratory has shown that when cultured in biological media (see Figure 1), macrolides could actually exert antimicrobial activity against *P.aeruginosa* (3). This is achieved by an increased accumulation of macrolides inside bacteria due to increased permeability of the outer membrane in these media and to repression of the expression of efflux systems.

Objectives

Our aims were

- to characterize susceptibility to azithromycin (AZM) in *P.aeruginosa* collected from CF patients vs. patients suffering from healthcare associated pneumonia, using in parallel CA-MHB and RPMI-1640.
- to specifically look for mutations in the ribosomal binding site of macrolides in strains harboring elevated MICs.

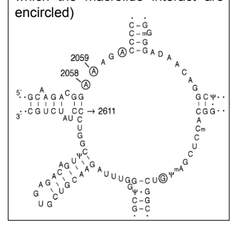
Materials & Methods

Bacterial isolates: PAO1 (fully sequenced genome) was used as reference strain. 333 strains from CF patients were examined in parallel to 48 strains isolated from patients suffering from healthcare-associated pneumonia and hospitalized in intensive care units (4). Two control strains originating from PAO1, kindly provided by RL Marvig (Technical University of Denmark, Lyngby, Denmark), were used as positive control. They contain plasmids encoding the mutated wild-type rRNA operons at two positions: A2045G and C2598T (2).

Susceptibility testing: MIC were determined by microdilution in cation-adjusted Mueller-Hinton broth (following CLSI recommendations), and Roswell Park Memorial Institute medium (RPMI-1640), as previously described (3). P.a. ATCC 27853 used as quality control strain. AZM was purchased from Teva, Plantex, Israel.

Sequencing of 23S ribosomal RNA gene: The gene corresponding to the domain V of 23S subunit of bacterial ribosome (625 bp; macrolide target) was amplified by PCR, purified and sequenced, focusing on regions were mutations were previously described (A2058G, A2059G and C2611T (5) in the 23S subunit of *E.coli,* corresponding to positions 2045, 2046 and 2598 in *Pseudomonas* species; see Figure 2).

Figure 2: Secondary-structure model of the peptidyl transferase center in domain V of 23S rRNA of *E.coli* (6) (nucleotides at which the macrolide interact are encircled)



Results

- CF strains are more resistant to macrolides in both RPMI 1640 and CA-MHB compared to pneumonia strains as shown in Figure 3. This suggests that some sort of resistance mechanism may have been selected among CF strains.

Figure 3: MIC distributions of AZM in RPMI 1640 and CA-MHB

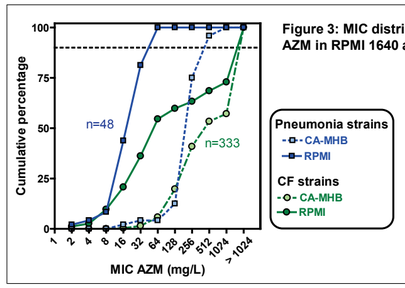
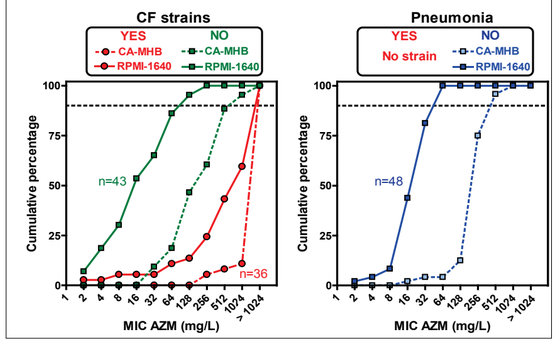


Figure 4: MIC distributions of AZM in CA-MHB and RPMI-1640 for CF strains and pneumonia strains presenting (YES) or not (NO) mutations on one of the three specific nucleotides on domain V of rRNA



- Mutations in domain V of 36 CF strains with elevated MICs were detected in three sites. Mutations in the first two sites (2045 and 2046) globally cause higher resistance in RPMI-1640 (Table 1).
- No mutations were detected in 48 strains collected from patients suffering from pneumonia.
- CF strains presenting no mutation on either of these three sites are significantly more susceptible than those with mutations (Fig 4).

Table 1: MICs values of AZM in CA-MHB and RPMI-1640 for PAO1, PAO1-pMES-23S(A2045G) (2), PAO1-pMES-23S(C2598T) (2) and CF strains presenting mutations

Strain	MIC of AZM in CA-MHB (mg/L)	MIC of AZM in RPMI-1640 (mg/L)
PAO1	**128**	**8**
Mutation : A2045G		
Control strain : PAO1-pMES-23S(A2045G)	**1024**	**256**
PA929	>1024	>1024
PA948	>1024	>1024
PA954	>1024	>1024
PA1024	>1024	>1024
PA1051	>1024	512
PA1062	>1024	>1024
PA1064	>1024	>1024
PA1065	>1024	>1024
PA1111(A2045T)	>1024	>1024
Mutation : A2046G		
PA941	>1024	256
PA942	>1024	1024
PA1001	>1024	>1024
PA1007	>1024	>1024
PA1012	>1024	1024
PA1074	>1024	>1024
PA1151	>1024	>1024
PA1154	>1024	64
PA1008(A2045T)	>1024	>1024
PA1009(A2045T)	>1024	1024
PA1036(A2045T)	>1024	>1024
PA1037(A2045T)	>1024	512
Mutation : C2598T		
Control strain : PAO1-pMES-23S(C2598T)	**512**	**64**
PA928	>1024	>1024
PA999	>1024	64
PA1007	>1024	1024
PA1020	>1024	1024
PA1021	>1024	1024
PA1027	>1024	512
PA1028	>1024	512
PA1066	>1024	512
PA1071	>1024	1024
PA1102	>1024	512
PA1214	>1024	128
PA1243	>1024	256
PA1274	>1024	256
PA1069(C2598G)	>1024	512
PA1110(C2598G)	>1024	64

Conclusions

- Mutations in the 23S subunit of bacterial rRNA could explain the high MICs of AZM recorded for strains coming from CF patients.
- Mutations in positions 2045 and 2046 confer higher resistance levels than those in 2611, probably because these two positions constitute the macrolide binding site while mutations in third site may rather alter the conformation of the center (7).
- Even though these mutations should not compromise the anti-inflammatory or anti-biofilm properties of macrolides, they raise concern over the prolonged use of this class of antibiotics.
- These findings may support the evaluation of the beneficial effects of ketolides in CF patients, as these also bind to domain II of 23S rRNA and therefore keep activity on macrolide-resistant strains.

A copy of this poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters.htm

References

1. Cystic Fibrosis Foundation. 2001. Patient registry 2001. Annual data report. Bethesda, MD.
2. Marvig *et al*, 2012, Mutations in 23S rRNA confer resistance against azithromycin in Pseudomonas aeruginosa. Antimicrob Agents Chemother, 56:4519–4521.
3. Buyck *et al*, 2012, Increased susceptibility of Pseudomonas aeruginosa to macrolides and ketolides in eukaryotic cell culture media and biological fluids due to decreased expression of oprM and increased outer-membrane permeability. Clin Infect Dis, 55:534-542.
4. Riou *et al*, 2010, In vivo development of antimicrobial resistance in Pseudomonas aeruginosa strains isolated from lower respiratory tract of intensive care unit patients with nosocomial pneumonia and receiving antipseudomonal therapy. Int J Antimicrob Ag, 36:513-522
5. Vester & Douthwaite, 2001, Macrolide resistance conferred by base substitutions in 23S rRNA. Antimicrob Agents Chemother, 45: 1–12.
6. Gutell *et al*, 1994, Lessons from an evolving rRNA: 16S and 23S rRNA structures from a comparative perspective. Microbiol Rev, 58:10–26.
7. Douthwaite *et al*, 2000, Macrolide-ketolide inhibition of MLS-resistant ribosomes is improved by alternative drug interaction with domain II of 23S rRNA. Mol Microbiol, 36:183-93

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