Pseudomonas aeruginosa is the main microorganism causing chronic respiratory tract infections in cystic fibrosis (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. Macrolides are deemed to be ineffective against P. aeruginosa, showing high MICs in conventional broth. However, our laboratory has shown that when cultured in biologically-relevant media (see Figure 1), macrolides could actually exert antimicrobial activity against P. aeruginosa (2). 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.

It is therefore not surprising that mutations in the macrolide target (domain V of 23S rRNA) could occur in P. aeruginosa isolated from CF patients (3).

Aims

Our aims were:

- to characterize susceptibility to azithromycin (AZM) in P. aeruginosa isolates collected from CF patients (potentially pre-exposed to AZM) vs. intensive care patients with healthcare associated pneumonia (HAP; no AZM pre-exposure), using in parallel CA-MHB and RPMI-1640.
- to explore the presence of mechanism of resistance to macrolides (mutations in domain V of 23S ribosomal RNA, macrolide site of action) in CF isolates vs HAP isolates.

Materials & Methods

Bacterial isolates: we used

- 333 isolates from CF patients
- 48 isolates from HAP patients and hospitalized in intensive care units (4).
- ATCC 27853 as a quality control for susceptibility testing.

PAO1 (fully sequenced genome) as reference strain and a derivative thereof containing a plasmid encoding the mutated (A2045G) rRNA operon (3) kindly provided by RL Marvig (Technical University of Denmark, Lyngby, Denmark). This plasmid was extracted and used as a backbone to introduce other mutations found in Table 1.

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 (2). AZM was purchased from Teva, Plantex, Israel.

Sequencing of 23S ribosomal RNA gene: The gene corresponding to the domain V of 23S rRNA of P. aeruginosa (625 bp; macrolide target) was amplified by PCR, purified and sequenced, focusing on regions were mutations were previously described (A2045G, A2046G, and C2598T), which were previously described to cause resistance to AZM in P. aeruginosa (4). Table 1: The gene

References


Table 1: MIC values of AZM in CA-MHB and RPMI-1640 for PAO1, PAO1-pMES-23S(A2045G) and PAO1-pMES-23S(C2598T) (or other mutations) and CF isolates presenting mutations

1. CF isolates were more resistant to AZM in both RPMI-1640 and CA-MHB compared to HAP isolates (Figure 3). This suggests that some sort of resistance mechanism may have been selected among CF strains.

2. Mutations were detected in 46% of CF isolates on 3 specific positions (A2045G, A2046G, and C2598T), which were previously described to cause resistance to AZM in P. aeruginosa (3). Table 1: The gene

3. No mutation was detected in all 48 HAP isolates.
4. Mutations in the 2 first sites (2045 and 2046) globally cause higher resistance as measured in RPMI-1640 (Table 1)

Table 1: MIC values of AZM in CA-MHB and RPMI-1640 for PAO1, PAO1-pMES-23S(A2045G) and PAO1-pMES-23S(C2598T) (or other mutations) and CF isolates presenting mutations

5. CF strains presenting no mutation on either of these 3 sites were significantly more susceptible than those harboring mutations (Figure 4).

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