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Paper Poster Session II

Focus: Acinetobacter, Pseudomonas and other nonfermenters

Mutations in 23S rRNA among *Pseudomonas aeruginosa* isolates from cystic fibrosis patients contribute to higher resistance to macrolides

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

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Objectives: *Pseudomonas aeruginosa* is a major cause of respiratory tract infections in Cystic Fibrosis (CF) patients older than 25 years. Beside from conventional antipseudomonal treatments, these patients also receive azithromycin by oral route for anti-inflammatory properties. However, we reported that azithromycin displays antibacterial effects on *P. aeruginosa* when tested in eukaryotic culture media or in bronchoalveolar lavage fluid (Buyck et al, CID 2012, 55:534-42). The aim of our study was to examine whether the prolonged exposure of CF patients to low concentrations of azithromycin may select for mutations causing high level macrolide resistance even in eukaryotic media.

Methods: Bacterial strains: PAO1 (fully sequenced genome) was used as reference strain. 38 strains isolated from CF patients were examined in parallel to 15 strains isolated from patients suffering from healthcare pneumonia hospitalized in intensive care units (ICU). Susceptibility testing: MICs were measured by microdilution in cation-adjusted Mueller-Hinton broth (following CLSI recommendations) and Roswell Park Memorial Institute medium (RPMI-1640). The gene corresponding to the domain V of 23S subunit of bacterial ribosome (625 bp; macrolide target) was amplified by PCR, purified and sequenced.

Results: Point mutations were identified in 40 % of the strains isolated from CF patients, affecting 3 specific nucleotide positions in domain V (A2058G, A2059G, C2611T [*Escherichia coli* numbering]), all of which having been previously described as causing azithromycin resistance in *P. aeruginosa* (Marvig et al, AAC 2012; 56:4519-21). In contrast, no mutation was found in strains collected from ICU patients. Table 1 shows MIC_{50} and MIC_{90} of azithromycin in CA-MHB and RPMI-1640. MICs were low in RPMI-1640, and similar for all isolates with no mutation whether originating from CF or ICU patients. In contrast, isolates with mutations were highly resistant whatever the medium used for testing.

Conclusions: Mutations in macrolide target are frequent in *P. aeruginosa* isolated from CF patients, possibly related to frequent treatments by oral azithromycin that may expose bacteria to low, subinhibitory concentrations. They cause high level resistance, reversing the enhancing effect of eukaryotic media on the antipseudomonal activity of azithromycin.

		Azithromycin MIC distributions (mg/L)			
		in CA-MHB		in RPMI-1640	
Origin of strains	23S rRNA sequencing	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
CF isolates	mutation (n=20)	>1024	>1024	1024	>1024
	no mutation (n= 18)	256	1024	16	64
ICU isolates	no mutation (n=15)	256	512	32	64