Colistin sulfate/tobramycin combination is superior for killing biofilm P. aeruginosa than monotherapy in vitro

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Here we investigated the efficacy of different antibiotics to kill P. aeruginosa in biofilms. Strain PAO1 was grown in the Calgary device to 8.9 × 107 CFU. At MICs, colistin sulfate, colistimethate, tobramycin or colistin sulfate/tobramycin reduced bacterial numbers to 6.5 × 104, 1.1 × 105, 1.2 × 105 and 3.5 × 104 CFU/ml, respectively. At 2 × MICs numbers were reduced to 3.9 × 104, 4.6 × 104, 7.0 × 104 and 4.0 × 102 CFU/ml, respectively. In flow cells 4-day-old GFP-labeled PAO1 biofilms were incubated for 24 and 48 h without and with addition of 10 × MICs of colistin sulfate, tobramycin or colistin sulfate/tobramycin to the medium, and the bacterial killing rate was determined using propidium iodide and the COMSTAT software. Addition of colistin sulfate, tobramycin or colistin sulfate/tobramycin killed 84.2%, 8.4% and 99.5% of P. aeruginosa after 24 h, and 77.9%, 35.5% and 98.8% after 48 h, respectively. Three-dimensional fluorescence imaging of the P. aeruginosa biofilm incubated with the various antibiotics allowed localization of dead and surviving bacteria in the ‘mushroom’ structures: while colistin sulfate killed predominantly the stalk population, tobramycin killed the cap population. The colistin sulfate/tobramycin combination killed both populations equally. Thus, colistin sulfate and tobramycin allow efficient killing of P. aeruginosa and suggest that simultaneous combination therapy in CF patients is more efficient than alternative single antibiotic therapy with these drugs to combat lung disease in CF. Supported by Grüenthal, Aachen, Germany.

Resistant in cystic fibrosis Pseudomonas aeruginosa strains is not mediated by common acquired antibiotic resistance genes

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Objectives: Beta-lactamases (BL) and aminoglycoside-modifying enzymes (AME) are important acquired ABR mechanisms in P. aeruginosa. This study analyzes the relation between the presence of ‘Antibiotic Resistance Genes’ (ARGs) and the ABR phenotype in CF and non-CF strains.

Methods: 328 unrelated P. aeruginosa clinical CF (43), non-CF (142), animal (63) and environmental (55) strains, collected in 69 localities on 5 continents and characterized by, amongst others, FAFLP fingerprinting were PCR-screened for the presence of 23 ARGs (15 coding for BLs, 8 for AMEs). MIC values for 21 antibiotics were determined by the VITEK 2 Advanced Expert System. The oprD gene was screened for ‘defective oprD mutations’ (DOMs) that confer carbapenem resistance.

Results: Fifty-eight ARGs were detected in clinical non-CF isolates. Surprisingly, CF isolates exhibited no ARGs at all, whilst showing a considerable level of ABR. Efflux pumps and biofilms cause broad spectrum ABR and are more common in CF isolates. Most of the clinical non-CF strains showed either a moderate or a very high ABR mediated ABR. 21 distinct DOMs mediated resistance to MER in 22 clinical strains, including 7 CF strains. There was a satisfactory correlation between ARG detection and the ABR phenotype.

Conclusion: The detection of ARGs through PCR has potential to generate timely but partial information regarding ABR in non-CF P. aeruginosa strains.

MRSA and macrolide resistant Staphylococcus aureus colonization rates in a Turkish CF center

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Introduction: Increasing prevalence of MRSA and macrolide resistant S. aureus in CF patients is an emerging problem. This study is aimed to determine the rate of MRSA and macrolide resistant S. aureus colonization in our CF patients over time.

Methods: A cross-sectional microbiological evaluation of S. aureus isolated from CF patients attending our center between 01/2005–12/2008 was undertaken. Antibiotic susceptibilities were determined by CLSI disk diffusion method. The prevalence of MRSA, multiresistant MRSA and macrolide resistant S. aureus were determined.

Results: The rate of S. aureus isolation was 39.4% in 2005, 46.5% in 2006, 57.9% in 2007 and 61.7% in 2008. The prevalence of MRSA and macrolide resistant S. aureus positive patients were 6.06%, 41.4% in 2005, 6.4%, 30.5% in 2006, 5.2%, 28% in 2007 and 4.9%, 26.9% in 2008, respectively. Among a total of 21 MRSA colonized patients, 8 (38.1%) were chronically colonized and six of them had also intermittent MSSA colonization. The remaining 13 MRSA cases were predominantly colonized with MSSA during their follow-up. Two of the chronically MRSA colonized cases had died. Among a total of 72 MRSA isolates, 68% were resistant to erythromycin, 72.2% to ciprofloxacin, 75% to gentamicin and 6.9 to TMP-SXT. Multidrug resistant MRSA was detected in 8 patients (38.1%).

Conclusion: Although MRSA colonization of CF patients is limited in our unit, multidrug resistant MRSA phenotype constitutes a significant proportion, thus necessitating adherence to infection control policies. Macrolide resistance although exhibiting a decreasing trend overtime, is of considerable importance and could be attributed to the increased use of macrolides especially in children.