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Department of Clinical Microbiology

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Collateral antibiotic synergism in multidrug-resistant *Pseudomonas aeruginosa*

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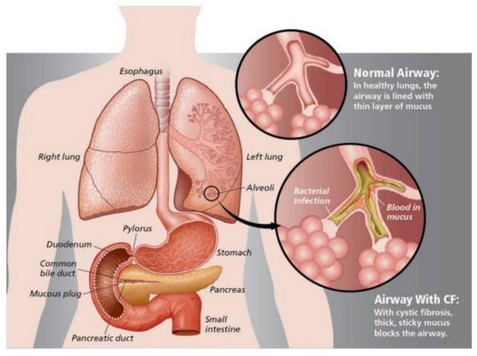
Background

Chronic lung infections with *Pseudomonas aeruginosa* show high morbidity and mortality rates in individuals with cystic fibrosis (CF) [1]. Eradication of chronic lung infections is challenging due to the high tolerance towards antibiotics and the emergence of multidrug-resistance (MDR).

Therefore, novel strategies improving the response to existing antibiotics, even those that display high rates of resistance, may successfully meet the need for new antibacterial therapies. A new approach for treating multidrug-resistant (MDR) pathogens is combining two or more antibiotics with different targets. It has been shown that some antibiotics shows synergistic effects when combined and thereby increases the killing of otherwise resistant pathogens[2].



An anatomical model of the airways with and without cystic fibrosis.

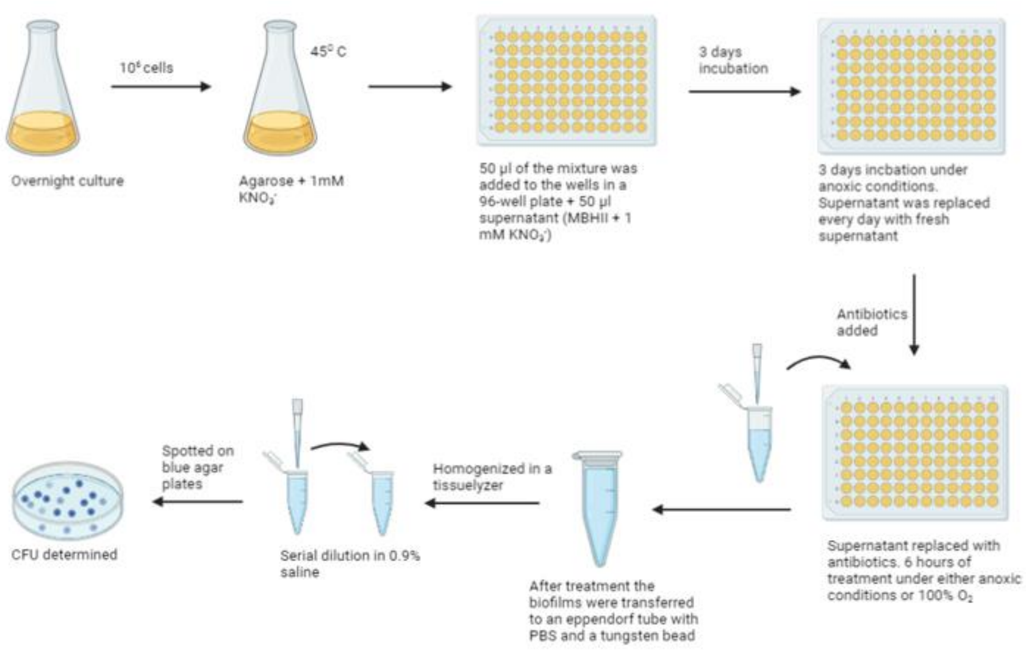
It has also been shown that applying O_2 to CF isolates makes the bacteria metabolically active again and therefore increases the antibiotic activity [3].

Aim

The aim of this study was to test relevant antibiotics that may lead to synergy in clinical MDR *P. aeruginosa* isolates.

Methods

We studied synergy on six clinical MDR *P. aeruginosa* isolates that were resistant to 14 different antibiotics. The isolates were isolated from sputum from CF patients with chronic lung infections.
All of the 6 isolates were screened for synergy with 31 combinations of relevant antibiotics using the microtiter checkerboard method according to EUCAST guidelines.
One of the 31 combinations was tested on agarose-embedded biofilms on all 6 isolates + the reference strain PAO1. During the treatment the biofilms were either treated under anoxic conditions (0% O_2) or under normobaric conditions (100% O_2, NBOT).



A schematic drawing of the biofilm assay.

Reference

(1) Z. Li et al., 'Longitudinal development of mucoid Pseudomonas aeruginosa infection and lung disease progression in children with cystic fibrosis', Jama, vol. 293, no. 5, pp. 581– 588, 2005.
(2) Acar JF. Antibiotic synergy and antagonism. Med Clin North Am 2000; 84: 1391–406.
(3) P. Ø. Jensen et al., Improving antibiotic treatment of bacterial biofilm by hyperbaric oxygen therapy: Not just hot air. Elsevier B. V., pp. 2590-2075, 2019.

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Results

Combinations of antibiotics show collateral sensitivity in MDR *P. aeruginosa* isolates

Combined effect of antibiotics on clinical CF isolates						
	I	II	III	IV	V	VI
Antibiotic combinations						
TGC+CST						
TGC+AMK						
AMK+ATM						
CTX+AMK						
AMK+CAZ						
AMK+IPM						
AMK+MEM						
TZP+AMK						
AMK+CIP						
ATM+TOB						
CTX+TOB						
CAZ+TOB						
IPM+TOB						
MEM+TOB						
TZP+TOB						
TOB+CIP						
AMK+CST						
GEN+CST						
TOB+CST						
IPM+CST						
MEM+CST						
CAZ+CST						
CTX+CST						
ATM+CST						
TZP+CST						
CST+CIP		N/A				
RIF+CST						
CAZ+CIP						
MEM+CIP						
ATM+CIP						
CST+AZM						

Schematic table of the 31 combinations of relevant antibiotics and their combined effect on the six MDR isolates. Green = collateral sensitivity, red = no collateral sensitivity. The combination of MEM and CST is highlighted because they showed collateral sensitivity in all six isolates. N/A = not determined.

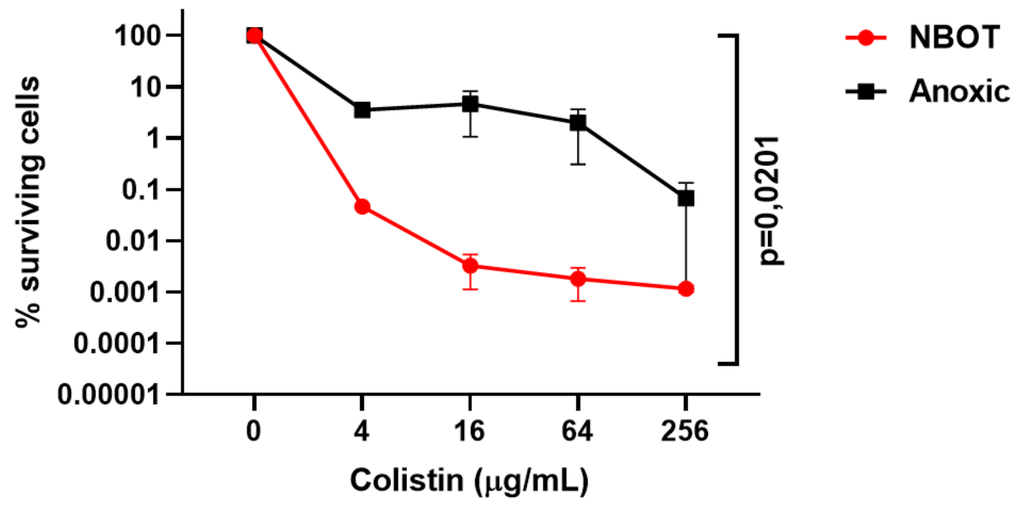
NBOT increases biofilm susceptibility towards the combination of colistin and meropenem

Biofilm isolates treated with colistin (0-256µg/ml) in combination with meropenem				
Isolates	Meropenem concentrations (µg/ml)			
	0	4	16	64
I	X			
II	X	X	X	
III		X		X
IV				X
V			X	
VI	X	X	X	
PAO1	X		X	

X = p-value < 0.05, significant difference between anoxic and NBOT. PAO1 was used as reference.

Representative CF isolate biofilm

Isolate IV - 64µg/ml MEM



CF isolate IV treated with CST and MEM in combination under either anoxic or NBOT conditions. P-value < 0.05 indicates significant difference.

Conclusion

We demonstrated synergy in MDR *P. aeruginosa* isolates from individuals with CF. Particularly, the combination of CST and MEM showed synergy during planktonic conditions.
We also demonstrated that by applying NBOT to the treatment of the biofilms, the killing was increased.