In vitro study of antibiotic combinations efficacy on multiresistant *Achromobacter xylosoxidans* strains

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Introduction

- ***Achromobacter xylosoxidans*** is an emerging Gram-negative opportunistic pathogen in **cystic fibrosis (CF) patients**[1].
- **These infections are difficult** to treat due to the **natural resistance** to a wide range of antibiotics[2]. Moreover, **acquired resistances** are frequent in CF thus **limiting therapeutic options**[3].
- The reasons for this emergence are still unknown and the therapeutic guidelines are very limited[4].

Purpose

To determine the efficacy of **antibiotic combinations** on **multiresistant *A. xylosoxidans* isolates from non-CF and CF patients**.

Material & Methods

Five *A. xylosoxidans* strains isolated from non CF patients were used in this study (NCF1, NCF2, NCF3, NCF4 and NCF5 strains) and three *A. xylosoxidans* strains isolated from the sputum of a CF patient at different steps of treatment (CFa, CFb and CFc strains) were used as well.

The study was performed according to these following steps :
1.MICs were determined for all strains by broth microdilution method (*Table 1*).

2.Screening of different combinations used in clinic[5] and unconventional combinations including rifampicin or piperacillin was performed by **checkerboard** (*Table 2*). Fractional inhibitory concentration index (FIC_i) values were determined according to the following equation: $FIC_i = FIC(A) + FIC(B) = \frac{MIC\ (A+B)}{MIC(A)} + \frac{MIC(A+B)}{MIC(B)}$

The FIC_i was interpreted as follows : $FIC_i \geq 0.5$ synergy, $0.5 < FIC_i > 2$ additivity, $FIC_i \geq 2$ antagonism.

3.Time-Kill curves (TKC) were performed with the promising combinations over CFa and CFc strains (*Fig. 1&2*).

Results

1. Susceptibility test of single antibiotics

Table1. MICs results for different *A. xylosoxidans* isolates

	Breakpoints		Non CF strains					CF strains		
	S	R	NCF1	NCF2	NCF3	NCF4	NCF5	CFa	CFb	CFc
Tobramycin (TOB) [a]	≤ 4	> 4	4	1	16	8	8	64	256	64
Ciprofloxacin (CIP)[a]	≤ 0.5	> 0.5	0.25	0.5	1	4	1	4	2	4
Colistin (CST)[a]	≤ 2	> 2	4	<1	2	1	2	8	16	16
Meropenem (MER)[a]	≤ 2	> 8	<0.25	<0.25	<0.25	<0.25	<0.25	0.5	64	128
Piperacillin (PIP)[a]	≤ 16	> 16	0.125	0.5	0.5	2	0.5	2	512	256
Chloramphenicol (CHL)[b]	≤ 8	> 8	8	8	8	8	8	4	4	4
Minocycline (MIN)[c]	≤ 16	> 16	0.25	0.25	1	1	1	1	1	2
Rifampicin (RIF)[c]	≤ 8	> 8	64	64	64	64	64	64	64	64

[a]According to the EUCAST breakpoints for *Pseudomonas aeruginosa*. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, 2018.
[b]According to the EUCAST breakpoints for *Enterobacteriacea*
[c]According to the BSAC breakpoints (Version 11.1 May 2012) for *Escherichia coli*

- All strains were susceptible to minocycline and chloramphenicol but resistant to rifampicin.
- 3 different resistance profiles were identified:
  - NCF1 and NCF2 strains were susceptible to all tested antibiotics.
  - NCF3, NCF4, NCF5 and CFa strains were resistant to ciprofloxacin and aminoglycosides.
  - CFb and CFc strains were resistant to all tested antibiotics.
  - ➢ **NCF1, NCF3, Cfa, CFb and CFc strains were selected for checkerboard due to their different resistance profiles**

2. Screening of antibiotics combinations

Table2. Effect of the antibiotic combinations

	Non CF strains		CF strains		
	NCF1	NCF4	CFa	CFb	CFc
MIN/CHL	0.31	0.53	0.75	0.63	0.63
MER/CIP	*ND*	*ND*	1.5	1	0.75
TOB/PIP	0.13	1.25	0.31	0.16	0.63
RIF/TOB	0.75	1	0.31	0.28	0.28
RIF/IMI	0.63	0.75	0.33	0.13	0.27
RIF/CST	0.63	0.75	0.25	0.28	0.56
RIF/PIP	1.25	1.5	0.16	0.31	2
PIP/CST	0.5	0.19	0.26	0.26	0.5

The values correspond to the minimum FIC_i for each combination.
Synergy : green cells - Additivity : yellow cells - Antagonism : red cells - ND : not determined.

- Tobramycin/Piperacillin and Piperacillin/Colistin combinations were synergistic.
- All combinations including rifampicin were synergistic against CF isolates.
- Minocycline/Chloramphenicol combination was synergistic against non-CF strains.
  - ➢ **Rifampicin/Tobramycin and Piperacillin/Colistin were investigated by TKC studies against CFa and CFc isolates.**

3. Time-kill curves (TKC) with the promising combinations

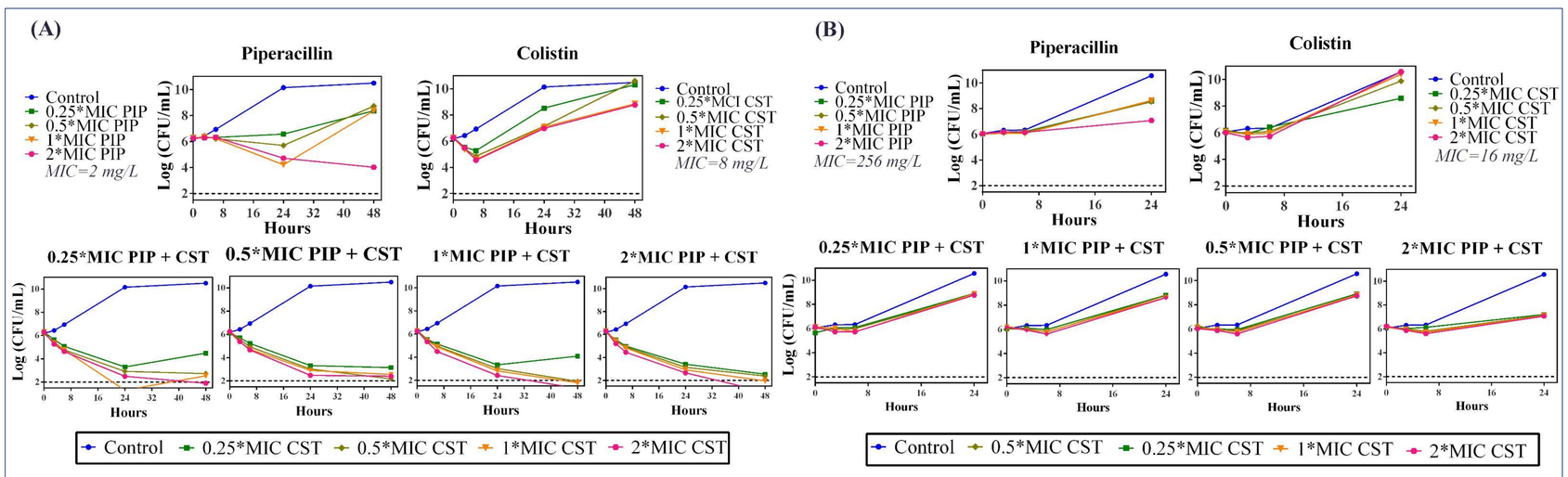

Fig 1. TKC of Piperacillin/Colistin combination for CFa (A) and CFc (B) strains

- A rapid killing activity was observed over the CFa strain from 0.25*MIC of each antibiotic in combination (Fig 1A).
- Piperacillin/colistin combination was not effective against the CFc strain (Fig 1B).

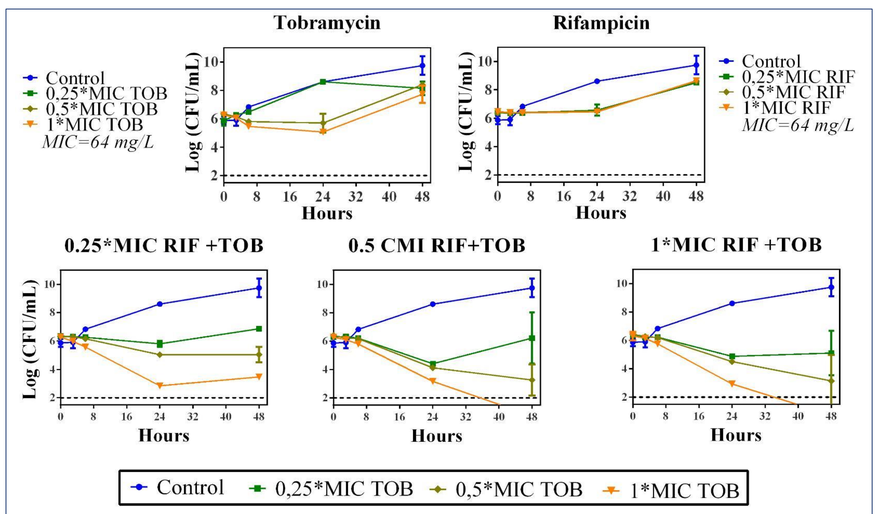

Fig 2. TKC of Rifampicin/Tobramycin combination for CFc strain

- A concentration-dependant killing effect was observed on the CFc strain.
- No regrowth was shown at the highest concentrations in combination.

Conclusion & Perspectives

- Two efficient antibiotic combinations against CF isolates were identified :
  - Piperacillin/Colistin is effective over strains that are susceptible to piperacillin
  - Rifampicin/Tobramycin allows to treat the infections due to resistant strains to a wide spectrum
  - The concentrations used in this *in vitro* study are not achievable in clinic due to their toxicity.
- *In vitro* studies are running on other *A. xylosoxidans* strains to confirm these results.
- Further investigations including *in vitro* dynamic time-kill study (hollow-fiber) on extended selection of strains and *in vivo* experiments are needed.

References

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