In vitro activity of polymyxin B alone and in combination against colistin-resistant *Acinetobacter Baumannii*

Vincent Aranzana-Climent[1,2], Alexia Chauzy[1,2], Nicolas Grégoire[1,2], Sandrine Marchand[1,2,3], William Couet[1,2,3], Julien M. Buyck[1,2]

1. Université de Poitiers, Poitiers, France 2. INSERM U1070, Poitiers, France 3. Service de Toxicologie et de Pharmacocinétique, CHU de Poitiers, France

vincent.aranzana.climent@univ-poitiers.fr

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Introduction

- Polymyxins are used as our last line of defence against multi-drug resistant *Acinetobacter baumannii* (1). However polymyxin resistance is emerging worldwide (2) and solutions are urgently needed.
- A possible solution could be to combine a polymyxin with another antibiotic in hope that the two substances synergise.
- There are currently two polymyxins currently on the market, colistin and polymyxin B. Colistin is administered as a prodrug while polymyxin B is administered as an active moiety, making its pharmacokinetics easier to predict.
- Also, in Europe only colistin is available for clinical use, making polymyxin B resistant strains less likely in absence of cross-resistance.
- Finally polymyxin B use seem to be associated with less nephrotoxicity than colistin use in humans (3).
- The purpose of this study was to identify promising polymyxin B based combinations to use against colistin resistant *A. baumannii*.

Materials/Methods

- Eleven *A. Baumannii* strains were tested: see details in **Table 1**.
- MICs were determined for all strains in duplicate by broth microdilution according to CLSI guidelines
- Checkerboards evaluating the effect of minocycline, rifampicin, aztreonam, chloramphenicol, fosfomycin and meropenem combined with polymyxin B were performed. For each well without bacterial growth, fractional inhibitory concentration index (FICI) values were calculated as follows:

$$FICI = FIC_A + FIC_{PMB} = \frac{[A]}{MIC_A} + \frac{[PMB]}{MIC_{PMB}}$$

([A] : concentration of the antibiotic associated with polymyxin B, MIC_A its MIC, [PMB] : concentration of polymyxin B, MIC_{PMB} its MIC)

- For each checkerboard plate, the minimum FICI ($FICI_{min}$) was determined and the $FICI_{min}$ values were averaged over the replicates. These $FICI_{min}$ were interpreted as follows: $FICI_{min} \leq 0.5$ synergy, $FICI_{min} > 4$ antagonism, $0.5 < FICI_{min} \leq 4$ no interaction.

Results & Discussion

Strain	Ref.	Description	CST (>2⁺)	PMB (>2*)	FOF (n.d.)	CHL (n.d.)	ATM (n.d.)	MEM (>4⁺)	MIN (>8*)	RIF (n.d.)
ATCC19606		Reference strain	0.25	0.5	128	64	16	0.5	0.06	1
CS01	(4)	Clinical isolate recovered from CSF of a patient treated for meningitis	0.5	0.25	512	128	32	64	4	>512
CR17	(4)	Isogenic derivative mutant of CR17; single amino acid substitution (Met16Lys) in PmrA	**128**	8	512	32	32	64	4	>512
062 D6	(5)	Clinical isolate recovered from bronchoscopy of a febrile patient	0.25	0.25	512	8	64	16	0.06	0.25
062 D7	(5)	Isogenic derivative mutant of 062 D6; with duplication of 30 nucleotides in PmrB	**256**	4	64	16	32	32	0.03	4
248	(6)	Clinical isolate recovered from pus of an ICU patient	0.5	0.5	256	64	128	8	0.25	2
249 pmrB	(6)	Isogenic derivative mutant of 248; single amino acid substitution (Pro233Ser) in PmrB	**128**	4	256	128	128	4	0.5	2
299	(6)	Clinical isolate recovered from bronchial secretion of an ICU patient	0.5	0.5	512	64	64	16	0.125	2
347 pmrB	(6)	Isogenic derivative mutant of 299; single amino acid substitution (Pro170Leu) in PmrB	**64**	4	512	32	128	8	0.25	2
Isac_ColiS	(7)	Clinical isolate recovered from pulmonary secretions of a pneumonic patient	0.5	0.5	256	64	32	32	0.25	0.125
Isac_ColiR	(7)	Isogenic derivative mutant of Isac_ColiS; single amino acid substitution in (Glu5Asp) PmrA	**128**	1	128	128	16	32	0.25	>512

Table 1. Description of *A. baumannii* isolates and MIC results. * Clinical breakpoint for resistance according to CLSI (18), ⁺ Clinical breakpoint for resistance according to EUCAST (17), n.d.: not determined. CST : colistin, PMB : polymyxin B, FOF : fosfomycin, CHL : chloramphenicol, ATM :aztreonam, MEM, meropenem, MIN : minocycline, RIF :rifampicin. In bold are colistin resistant strains.

Strain	Means of $FICI_{min}$					
	FOF	CHL	ATM	MEM	MIN	RIF
ATCC19606	0.50 (S)	0.61 (NI)	0.73 (NI)	0.88 (NI)	0.56 (NI)	0.50 (S)
CS01	0.88 (NI)	0.52 (NI)	0.69 (NI)	0.79 (NI)	0.81 (NI)	1.00 (NI)
CR17	0.22 (S)	0.63 (NI)	0.31 (S)	0.31 (S)	0.27 (S)	0.10 (S)
062 D6	0.71 (NI)	0.77 (NI)	0.70 (NI)	0.77 (NI)	0.67 (NI)	0.41 (S)
062 D7	0.64 (NI)	0.33 (S)	0.42 (S)	0.50 (S)	0.79 (NI)	0.12 (S)
248	0.69 (NI)	0.69 (NI)	0.52 (NI)	0.55 (NI)	0.55 (NI)	0.39 (S)
249 pmrB	0.45 (S)	0.44 (S)	0.43 (S)	0.38 (S)	0.40 (S)	0.08 (S)
299	0.31 (S)	0.63 (NI)	0.69 (NI)	0.50 (S)	0.53 (NI)	0.69 (NI)
347 pmrB	0.46 (S)	0.59 (NI)	0.31 (S)	0.28 (S)	0.44 (S)	0.09 (S)
Isac_ColiS	0.34 (S)	0.56 (NI)	0.66 (NI)	0.50 (S)	0.31 (S)	0.38 (S)
Isac_ColiR	0.41 (S)	0.52 (NI)	0.44 (S)	0.44 (S)	0.50 (S)	0.53 (NI)

Table 2: FICImin from checkerboard synergy testing. FICImin were determined by checkerboard; the values correspond to the mean of the minimal FICI values for each plate. S: Synergy, NI: No interaction. Green cells are indicating synergy.

- All strains showed high MICs for aztreonam, chloramphenicol and fosfomycin and 7/11 strains were resistant to meropenem.
- All strains were susceptible to minocycline, and all but 3 (CS01, CR17, Isac_ColiR) showed low rifampicin MICs.
- On all colistin resistant strains; using clinical concentrations of minocycline restored their susceptibility to polymyxin B, i.e. in presence of minocycline, the polymyxin B MIC was below 2mg/L
- Using clinical concentrations of rifampicin restored polymyxin B susceptibility of 4/5 colistin resistant strains, including CR17 which had a rifampicin MIC >512 mg/L. When polymyxin B and rifampicin were combined against CR17, 8 mg/L of rifampicin brought the polymyxin B MIC to 1 mg/L

Conclusion

- **Polymyxin B exhibited much better activity than colistin** against the studied colistin resistant strains.
- **Combining polymyxin B with minocycline or rifampicin show promising results**, especially on colistin resistant strains.
- These results have to be confirmed by performing experiments like *in vitro* time-kill experiments to study interaction concentration effect relationships more in depth and *in vivo* experiments.

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