Sequential Time-Kill Experiments to Characterize Adaptive Resistance to Polymyxins in Gram-Negative Bacteria Carrying *mcr-1*

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Introduction

- Colistin and polymyxin B (Polymyxins class) are used as last resort drugs to treat multidrug resistance (MDR) Gram-negative infections (1)
- Resistance to polymyxins are increasing and plasmid-carried resistance (MCR-1) in natural colistin susceptible Gram-negative bacteria (GNB) has been recently reported (2,3)
- In *E. coli* study, *mcr-1* mediated low-level of polymyxins resistance by encoded the expression of phosphoethanolamine in lipid A of bacterial cell membrane (4). Whether *mcr-1* could increase GNB resistance level has not been studied yet

Purpose

- To decipher heterogeneity of bacterial population (existing resistant subpopulation in total population) or adaptation to polymyxins resistance
- To describe, by an original approach of sequential time-kill experiments, the role of MCR-1 in the development of additional adaptive resistance to polymyxins in MDR Gram-negative bacteria.

Methods

- *Escherichia coli* J53, *Klebsiella pneumoniae* R2292 and their transconjugant *mcr-1* (J53_*mcr1* & KP_*mcr1*) were used in this study which were kindly provided by P. Nordmann

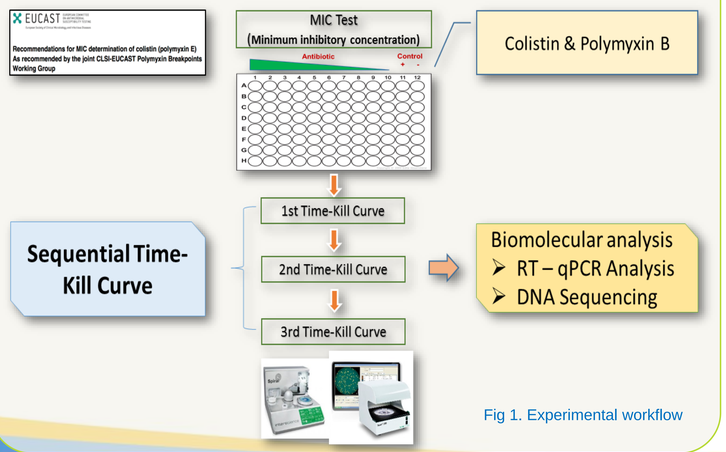

Fig 1. Experimental workflow

Methods

- The inoculum used in 2nd or 3rd time-kill curve (TKC) were taken from the previous TKC obtained from the regrowth bacteria at maximal antibiotic concentration
- 1st Time-kill study :
  - ✓ Concentrations: 0,25; 0,5; 1; 2 and 4*MIC
- 2nd and 3rd Time-kill study:
  - ✓ Concentrations: adapted based on the 1st and 2nd time-kill study, respectively.
- Analysis of heteroresistant subpopulations of bacteria by Population analysis profiles (PAPs) and MICs determination after TKC were performed as well

Results

Sequential Time-Kill Curves*

E. coli J53 — *E. coli* J53_*mcr-1*

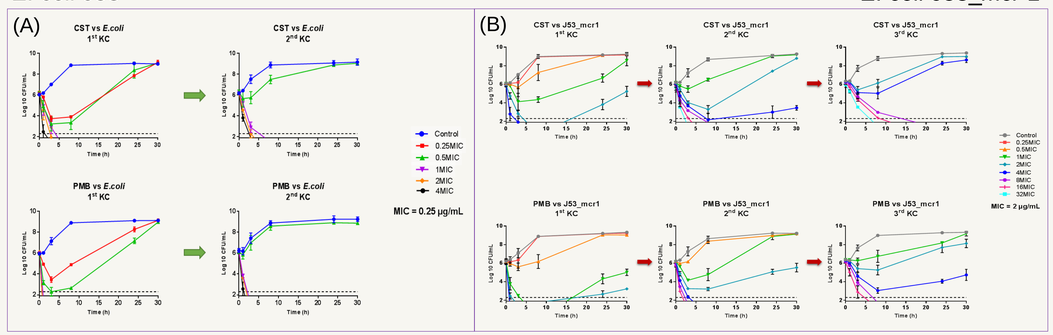

Fig 2. Time-Kill curves of colistin (CST) & polymyxin B (PMB) for *E.coli* J53 & *E.coli* J53 containing plasmid MCR-1 (J53_*mcr1*)

K. pneumoniae R2292 — *K. pneumoniae* R2292_*mcr-1*

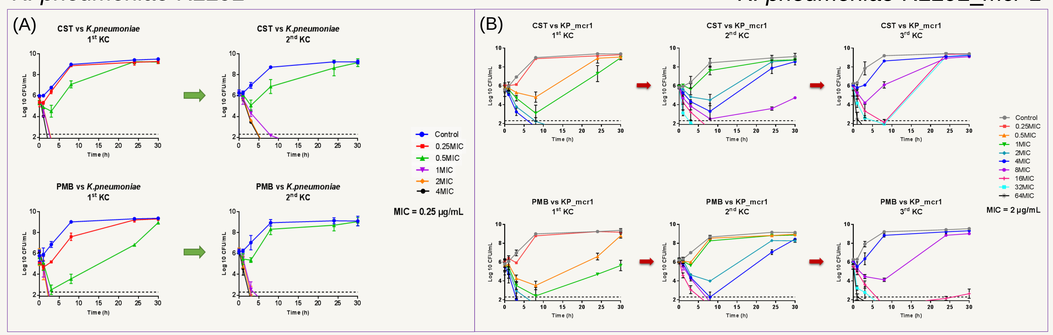

Fig 3. Time-Kill data of CST & PMB for *K. pneumoniae* & *K. pneumoniae* with *mcr-1* (KP_*mcr1*)

- ✓ Reference strains are not able to adapt the resistance to colistin & polymyxin B (only resistant subpopulation) (Fig. 2A & 3A)

*Data are presented as means and standard deviations after two times replications

Results

- ✓ The presence of MCR-1 leads to high-level polymyxins resistance (Fig. 2B, 3B & 4)

Strain	Colistin	Polymyxin B	
J53	0,25	0,125	
J53 - 1st TKC	0,25	0,25	
J53 - 2nd TKC	0,25	0,25	
J53_*mcr1*	2	2	LOW Level Resistance
J53_*mcr1* - 1st TKC	8	4	
J53_*mcr1* - 2nd TKC	16	8	
J53_*mcr1* - 3rd TKC	32	16	HIGH Level Resistance
KP	0,25	0,25	
KP - 1st KC	0,25	0,25	
KP - 2nd KC	0,25	0,25	
KP_*mcr1*	2	2	LOW Level Resistance
KP_*mcr1* - 1st TKC	16	8	
KP_*mcr1* - 2nd TKC	64	16	
KP_*mcr1* - 3rd TKC	512	128	HIGH Level Resistance

Fig 4. MICs (mg/L) confirmation after sequential Time-Kill Curve

- ✓ PAPs confirmed the absence of heteroresistant subpopulation since both antibiotics exhibited bacterial killing above 0.125 mg/L and 4 mg/L concentration for reference strain and their transconjugants respectively

Conclusion

Reference strains did not show adaptive resistance to polymyxins while the presence of MCR-1 facilitated the step-by-step adaptation. These findings suggest that plasmid MCR-1 favor selection of another resistance mechanism, which may lead to develop high resistance to polymyxins. Biomolecular studies are running to determine mechanisms involved.

References

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