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ASSESSMENT OF THE SELECTIVE EFFECT OF COLISTIN ON *MCR-1*-POSITIVE *ESCHERICHIA COLI* THROUGH *IN VITRO* CO-CULTURE TIME-KILL EXPERIMENTS.

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CONTEXT

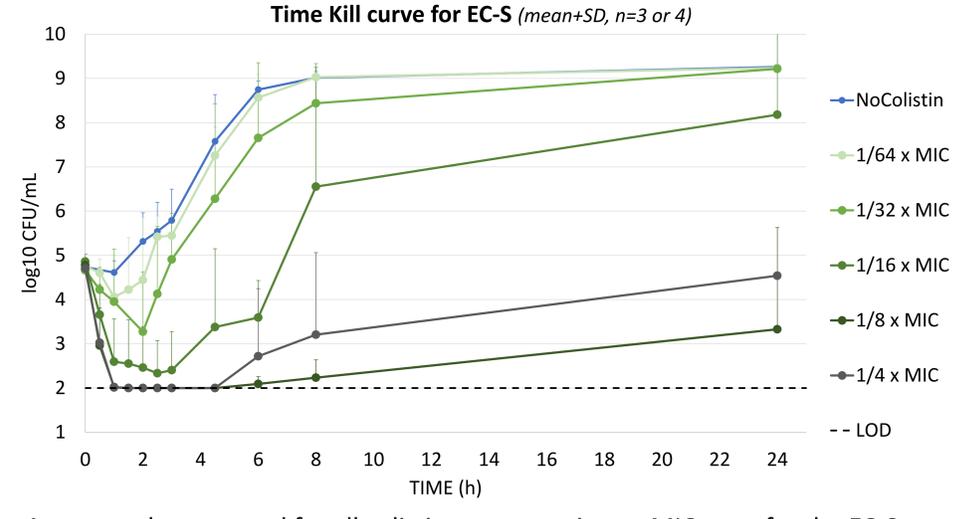
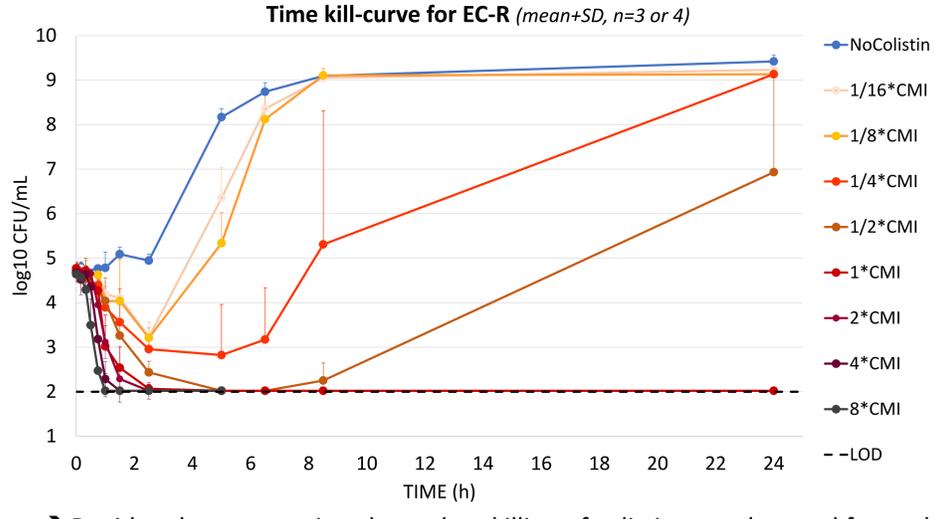
Colistin is widely used in food-producing animals, especially in pigs against digestive infections after weaning due to pathogenic *E.coli*. However, concerns about its use have been raised due to the discovery of plasmid-mediated resistance genes, *mcr*. Unexpectedly, we have not observed any significant selective effect of an oral colistin treatment in pigs harboring a *mcr-1* positive *E.coli* (named EC-R, MIC=8 mg/L) at a sub-dominant level within guts, despite a huge decrease of the total *E.coli* population during treatment [1].



In order to better characterize the mutant selection window and the apparent lack of selective effect of colistin, we carried-out several time kill curves (TKC) experiment with the EC-R strain and a sensitive *E.coli* strain (named EC-S, MIC=0.25 mg/L), either alone or in co-culture condition. A mechanistic PKPD model is under development to support the observed data.

SEPARATED TIME-KILL CURVES

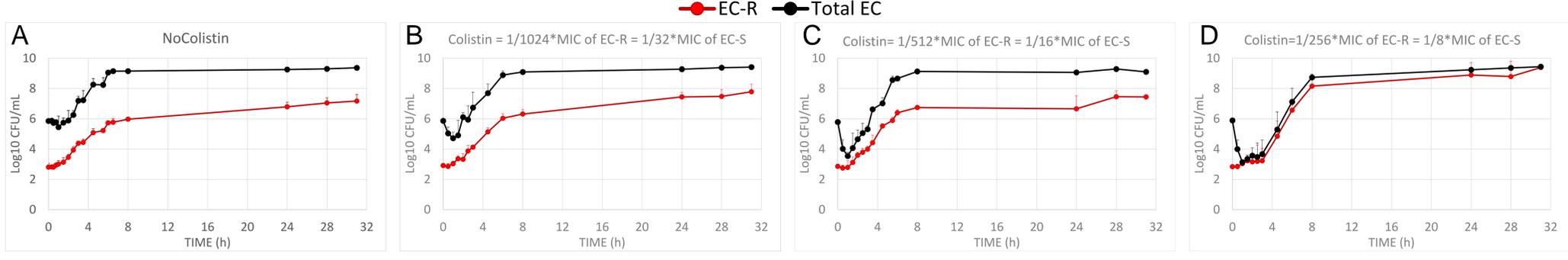
METHODS: Time kill-curves were first carried-out in separated tubes for EC-S and EC-R, with different concentrations of colistin as multiples of their respective MIC.



- Rapid and concentration-dependant killing of colistin was observed for each strain. A re-growth was noted for all colistin concentrations < MIC even for the EC-S
- These TKC are necessary to assess the response of each strain to colistin separately and inform the mechanistic PKPD model.

CO-CULTURE TIME KILL CURVES and PKPD MODEL

METHODS: Time kill-curves were performed in co-culturing condition, with EC-S at a dominant level (starting inoculum: 6 log₁₀ CFU/mL) and EC-R at a sub-dominant level (starting inoculum: 3 log₁₀ CFU/mL), as a model of *E.coli* population within pig guts. Total *E.coli* and EC-R were counted and differentiated thanks to selective agar plates.



→ After 24h, EC-R stayed at a subdominant level (~2 log₁₀ under total *E.coli* level) in graphs A - C, even when total *E.coli* (and therefore EC-S) concentrations decreased sharply between 0 and 4h (C). However, for the highest concentration (D), EC-R became dominant, i.e. equal to the total *E.coli* population.

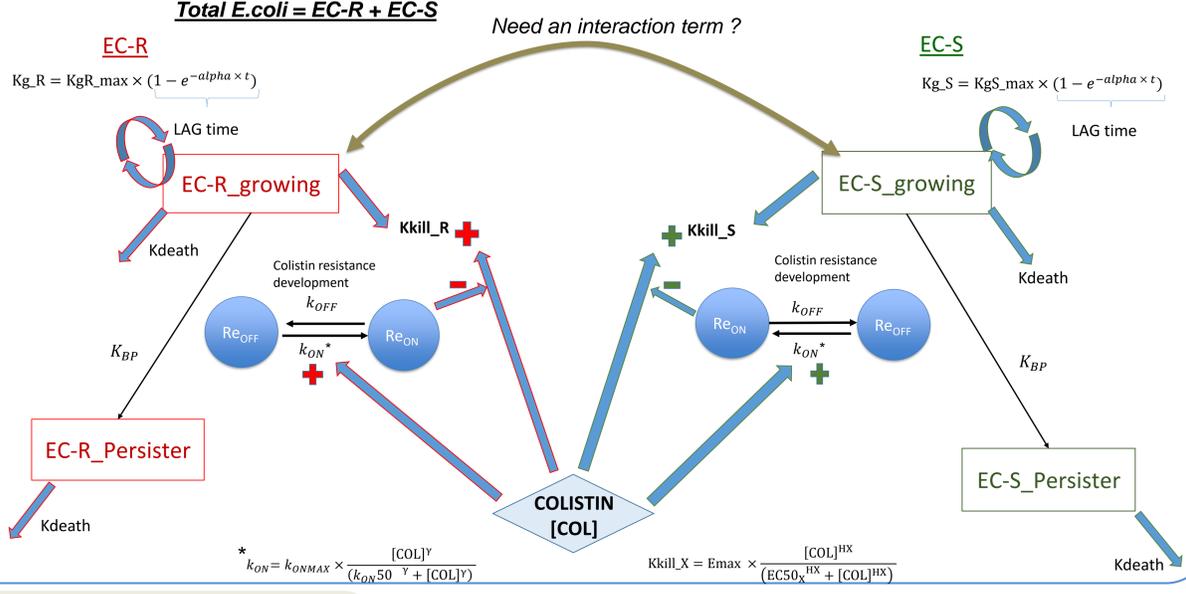
→ The range of colistin concentrations avoiding the selection window, i.e. targeting a significant decrease of the sensitive strains while keeping the resistant ones at sub-dominant level, seems very narrow

PKPD model : A previous semi-mechanistic PKPD model was adapted [2] and fitted to the EC-R, EC-S and total *E.coli* data from all TKC:

- Each strain is divided in growing (logistic growth) and persisters (non-growing, non-susceptible) sub-populations.
- Colistin killing is described by an E_{max} equation.
- The observed regrowth was described by an adaptative resistance model (Re_{OFF} and Re_{ON}) affecting the actual killing rate constant (Kkill)
- The resistance onset rate k_{ON} was determined by the colistin concentration through an E_{max} relationship

→ The PKPD model is able to fit the data from the separated TKC but failed to capture properly the co-culturing TKC data.

→ Need to add supplementary interactions terms between the 2 strains [3] ?



CONCLUSION

- The TKC in co-culture condition with sensitive/resistant strains is a mean to explore quantitatively the mutant selection window of an antibiotic
- Our experiments highlight the difficulty of finding an effective concentration that avoids the amplification of a resistant strain that would be present at a sub-dominant level
- The use of dynamic systems (chemostat/hollow-fiber) may be necessary to properly characterize the interactions between resistant and sensitive strains, and refine the mechanistic PKPD model

REFERENCES

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 [3] Lenhard, J. R et al., (2019). JAC, <https://doi.org/10.1093/jac/dkz247>

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