Investigation of PmrA/PmrB polymorphism leading to colistin sulfate resistance in *E.coli* O149 strain *in vitro* and in a clinical model of post-weaning diarrhea in piglets

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Abstract

The post-weaning diarrhea (PWD) is an important economical swine disease caused by *E.coli* O149. Usually, in Canada veterinarians use neomycin, an aminoglycoside antibiotic for the treatment of this disease but unfortunately, near 40% of these treatments are unsuccessful due to *E. coli* resistance to this antibiotic. Thereby, veterinarians use colistin sulfate (CS), for the treatment of PWD. CS binds to LPSs and leads to disruption of the outer cell membrane, leakage of intracellular contents and bacterial death. Mutation in the two components system PmrA/PmrB is the most frequently associated with CS resistance in *E.coli* by adding a L-Ara4N and/or phosphoethanolamine group reducing the LPS affinity for colistin.

Since CS is given to piglet to treat PWD, the main objective of this study was to investigate genetic polymorphism in the two components system PmrA/PmrB associated to CS resistance in *E.coli* isolates from *in vitro* assay and in *vivo* clinical model of PWD in piglets.

For the i*n vitro* assay, we used swine clinical *E.coli* strains resistant to neomycin isolated from 2008 to 2011. We created 22 mutants resistant to CS. MIC was determined by standard double dilution method and E-test and compare to the EUCAST breakpoint. For the *in vivo* assay, we followed the experimental infection strain (ETEC: F4) and commensal *E. coli* isolates through a post-weaning infection model.

The sequencing of the *pmrA* and *pmrB* genes from in vitro mutants showed 4 different mutations in *pmrA* gene and 5 in *pmrB* gene leading to a CS resistance phenotype. Eleven of the variants had a CS

resistant phenotype but no mutation in the *pmrA* and *pmrB* genes were identified. After *E.coli* confirmation of presumptive isolates from pigs in the PWD infection model, 9 isolates were confirmed as *E.coli* and one isolate was the ETEC: F4 challenge strain. The ETEC: F4 strain was the only one with a *pmrA* gene mutation. Out of the 9 *E.coli* isolates, only 3 had a *pmrB* gene mutation. The others 6 *E.coli* resistant isolates didn't have mutation in these two genes. By sequencing the *pmrA* and *pmrB* genes, we identified seven new genetic polymorphisms, 3 were in PmrA: A80V, N128I, S144G and 4 in PmrB: V87E, D148Y, D148V, T156M. We also found polymorphisms that have already been reported in other enterobacteriaceae namely, G15R in PmrA and V161G in PmrB. *In vivo E.coli* strains showed two polymorphisms G15R and T156M, in PmrA and PmrB respectively.

To our knowledge, this is the first study that reports a G53R mutation in PmrA and a T156M mutation in PmrB in *E.coli* isolates from an animal source, suggesting that CS resistance can be a public health concern knowing that this antibiotic is classified as a very high importance in human medicine in Canada. Furthermore, our results indicated that many *E.coli* isolates showed resistance to CS without genetic polymorphism in the PmrA/PmrB system, suggesting potential other(s) mechanism(s) of resistance. Further studies are needed to better understand the CS resistance acquisition and to reach a judicious use of this antibiotic in veterinary medicine.