Study of therapeutics strategies for piglets postweaning diarrhea: in vitro characterization of the colistin outcome in digestive tract, resistance and transfert of neomycin resistance in *E.coli* 0149 strains

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Neomycin, an aminoglycoside, is used at the farm level for the treatment of piglets postweaning diarrhea, with 40% of unsuccessfully treatment due to the antimicrobial resistance. To overcome this situation, veterinarians use colistin sulfate (CS) also called polymyxin E, but this antibiotic is not authorized in Canada, even if it seems to be effective against *E.coli*. It is also known that CS is not absorbed at intestine level, suggesting selection pressure on intestinal microflora. On the other side, resistance to colistin has been reported more frequently in countries where it is used. The described resistance to polymyxin is associated to a modification of the LPS core and the lipid A regions in the bacteria. For *Salmonella*, those modifications are associated to the two components system PmrA-PmrB. This system is known in *E.coli* but not yet reported to be implicated in colistin resistance. The neomycin resistance is linked to enzymatic modifications associated to genes located on plasmids.

This study has for objectives to investigate the fate of CS into pig's digestive tract, the acquisition of colistin resistance and study the transfer of neomycin resistance between *E.coli* strains and other enterobacteria. A simulated gastric fluid (SGF) was performed with a clinical CS dose. Samples were collect after precise time and concentration of CS was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). Another concentration of 32 µg of CS was used to evaluate antimicrobial activity after acetonitrile neutralization with evaporation. Antimicrobial activity assays were conducted by micro-dilution method on ATCC 25922 *E.coli*.

Results of LC-MS/MS showed a rapid degradation of CS in SGF, this degradation started quickly (from the 5th minute) and reached a maximum around 10 minutes with 50% of CS degradation. Samples from each time point showed stronger antimicrobial activity than non-degraded CS. Eight strains of *E.coli* O149 isolated from clinical cases (2008 to 2011) were used and antimicrobial susceptibility testing was performed by the disk diffusion method. E-test and a micro-dilution method were used to determine the minimal inhibitory concentration (MIC) of colistin and neomycin respectively. All tested strains had a MIC higher than 128 ppm for neomycin whereas MICs were between 0.064 to 0.128 ppm for colistin, indicating that all strains were resistant to neomycin but all susceptible to colistin. In these isolates, the antimicrobial resistance genes *aac(3)-IV*, *aphA1* and *aphA2* have been detected using PCR. Conjugation experiments are presently being performed. Colistin natural mutants (n=24) were created through serial passages on LB agar with 2.5xMIC. Sequencing of the PmrA-PmrB region of these mutants was performed to identify possible mutations and none were observed within this region. Investigation of colistin resistance associated with other DNA regions are currently under investigation.

This is the first study to show colistin instability at the gastric level and that degradation product are more potent than CS. Also we should be able to detect precisely the mutations leading to colistin resistance and trace the outcome of neomycin resistance.