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In vitro characterization of colistin resistance and transfert of neomycin resistance in *Escherichia coli* O149 strains

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Neomycin, an aminoglycoside, is use at farm level for *Escherichia coli* treatment of piglets postweaning diarrhea, with 40% of unsuccessfully treatment due to the antimicrobial resistance. To overcome this situation, veterinarians use colistin also call polymyxin E, but this antibiotic is not homologated in Canada, even if it seems to be effective against *E.coli*. 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 acquisition of colistin resistance and study the transfer of neomycin resistance between *E.coli* strains and other enterobacteria. *E.coli* O149 strains isolated from clinical cases (2008 to 2011) were used and susceptibility testing of strains 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 resistance to neomycin but all susceptible to colistin. In these isolates, neomycin antimicrobial resistance genes *aac(3)-IV*, *aphA1* and *aphA2* have been detected using PCR. Conjugation experiments are presently being performed. Colistin natural mutants (n=22) were created through serial passages on LB agar with 25xMIC. Sequencing of the PmrA-PmrB region of these mutants was performed to identify mutations. Three PmrA and eight PmrB mutations were for the first time reported in *E.coli* O149. Others mutants are still under investigation for other possible resistance pathway.

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