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P2473 Genetic characterization of an MCR-3-like producing Escherichia coli recovered from swine, Brazil

Laurent Poirel*[1 1], Nicolas Kieffer[1], Andrea Micke Moreno[2], Richard Chaby[3], Aude Breton[3], Pierre Tissieres[3], Patrice Nordmann[1]

[1]University of Fribourg, Medicine, Fribourg, Switzerland, *[2]Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, Sao Paulo, Brazil*, *[3]Institute for integrative biology of the cell, CEA, Saclay, France*

Background: The occurrence of plasmid-mediated colistin-resistant determinants in Enterobacteriaceae is of great concern since it jeopardizes the efficacy of that last-resort antibiotic. Since 2015, five different plasmid-mediated colistin resistance genes have been described, different from each other by 19% to 66% amino acids. To date, six *mcr-3* variants have been reported. Here we report a novel *mcr-3* variant detected in an *Escherichia coli* isolate recovered from a post-weaning diarrhea of a pig in Brazil that was previously treated with colistin.

Materials/methods: Screening of colistin-resistant isolates was performed from 126 different pigs in the state of Minas Gerais in Brazil. Colistin minimal inhibitory concentrations (MICs) were determined using broth microdilution method using cation-adjusted MH broth. The whole genome of the isolate was sequenced using Illumina MiniSeq system and reads were assembled using CLC genomic workbench.

The *mcr-3*-like gene variant was cloned into the arabinose-inducible $pBAD_b$ vector in order to determine the impact of the MCR-3 enzyme variant in the colistin susceptibility. Induction of $pBAD_b$ vector was performed in MH broth supplemented with 1% of L-arabinose. The lipopolysaccharide (LPS) of the MCR-3 producer was extracted and analyzed by mass spectrometry and compared to that of reference E. coli strains.

Results: Isolate I112 was positive for the *mcr*-3 gene and sequencing revealed that the corresponding protein shared 97% of amino-acid identity with the original MCR-3 variant identified. Induction of the $pBAD_b$-*mcr-3-like* plasmid conferred an MIC of colistin at 4 µg/ml whereas the non-induced clone had a MIC at 0.03 µg/ml. Molecular characterization showed that the *mcr-3-like* gene was located onto a

ca. 140kb conjugative IncA/C_2 plasmid. Analysis of the LPS of the MCR-3 producer showed an addition of a phosphoethanolamine group to the lipid A the same way as MCR-1 and MCR-2.

Conclusions: This study identified the first occurrence of an MCR-3-like resistance determinant in South America. We demonstrated the enzymatic function acting as a phosphoethanolamine transferase. This is one of the few demonstration deciphering the impact of such phosphoethanolamine transferase in modifying the LPS and ultimately impact the susceptibility to polymyxins.