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O0510 *Buttiauxella* spp. as progenitor of transferable *mcr-9*-like phosphoethanolamine transferases in *Enterobacteriaceae*

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Background: Emergence of transferable colistin-resistant MCR determinants in Enterobacteriaceae represents a threat. Nine distinct plasmid-mediated colistin resistance genes (*mcr-1* to *-9*) have been identified, differing from each other by 15% to 65% at the nucleotide level. They encode phosphoethanolamine (PEt) transferases adding a PEt group to the lipopolysaccharide (LPS) leading to resistance to polymyxins. The origins of some *mcr* genes have been identified, with *mcr-1* and *mcr-2* being from *Moraxella* spp. Here we identified *Buttiauxella* spp. being the likely source of the newly-identified *mcr-9* gene.

Materials/methods: MICs of colistin were determined using broth microdilution method using cation-adjusted MH broth. The *mcr-9* and *mcr-BG* genes were cloned into the arabinose-inducible $pBAD_b$ vector and expressed in *E. coli*. The LPS of *Buttiauxella gaviniae* strain CIP106356T and *E. coli* (pmcr-BG) was extracted and analyzed by mass spectrometry and compared to that of reference *E. coli* strains. Quantitative expression of *mcr* genes was measured by RT-PCR.

Results: *In silico* analysis identified a *mcr-9*-like gene from the genome of *B. gaviniae* strain CIP106356T. This *mcr-BG* gene was cloned and expressed in *E. coli* TOP10. A minor impact of the *mcr-BG* gene on susceptibility to polymyxins was observed once cloned and produced in *E. coli*, similarly to what was observed with *mcr-9* expressed in *E. coli*. A 8-fold increase in MIC of colistin was noticed for *E. coli* TOP10 producing MCR-BG (0.015 to 0.125 µg/ml) or producing MCR-9 (0.25 µg/ml) as compared to *E. coli* TOP10, while the MICs of colistin for MCR-1 recombinant strain was 4 µg/ml. The MCR-BG protein shares 84% identity with MCR-9, and 30-60% with the other plasmid-encoded MCR-type enzymes (MCR-1- to -8). Analysis of the LPS pattern of the MCR-BG-producing *E. coli* confirmed that a PEt group was added to the lipid A component of the LPS of *E. coli*, while no specific addition was observed when analyzing the *B. gaviniae* strain. Accordingly, qRT-PCR showed a lack of expression of *mcr-BG* in its original host.

Conclusions: *Buttiauxella* spp. species are likely the progenitor of MCR-9-like determinants, further underlining the origin of MCR-like determinants in Gram-negative bacteria.