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MCR-like protein from *Kosakonia sacchari*, an environmental Enterobacterales

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Sir,

The recent identification of plasmid-encoded enzymes conferring resistance to polymyxins, namely MCR enzymes, and their worldwide emergence in Gram-negative bacteria is a major threat in the battle against multidrug-resistant bacteria. MCR proteins function as lipid A phosphoethanolamine transferases (EptA) and are found among *Escherichia coli* and *Klebsiella pneumoniae*. To date, ten genes encoding MCR enzymes have been reported from human isolates, but to a large extent also from animal isolates, particularly from swine and cattle in relation to the significant colistin selective pressure in such farm production.

Wang et al. recently identified the *mcr-8* gene from a colistin- and carbapenem-resistant *K. pneumoniae* producing NDM-1 in China [1]. Since then, most of the MCR-8-producing isolates identified were *K. pneumoniae* isolates, some of these corresponding to successful clones (such as ST15) and often producing carbapenemases. The *mcr-8* gene has also been reported in *Raoultella* spp. and *E. coli* in chicken from China and Nigeria, in *Stenotrophomonas maltophilia* isolates recovered from sewage water of a poultry farm in China, and in *Klebsiella quasipneumoniae* isolates from pigs in China.

In silico analysis of the *mcr-8* nucleotide sequence using the GenBank database identified genes sharing significant identities in the chromosome of different bacterial species, including *Stenotrophomonas* spp., *Pectobacterium* spp., *Xanthomonas* spp. and *Kosakonia* spp. The most closely related gene, that we named *mcr-kos*, was identified in the chromosome of *Kosakonia sacchari* strain BO-1 (GenBank accession no. CP016337) encoding a putative protein sharing 70% amino acid identity with MCR-8, MCR-9 and MCR-10, 44% with MCR-7, 41% with MCR-3 and MCR-5, 40% with MCR-4, and 35% with MCR-1 and MCR-2. No putative mobile element could be identified by analysing the 5-kb long *mcr-kos* upstream and downstream sequences, suggesting an intrinsic location rather than a recent acquisition. No plasmid feature could be identified by analysing the whole genome sequence. Altogether, these features likely rule out recent acquisition of the gene in *K. sacchari*. Noteworthy, *Kosakonia* species, members of the Enterobacteriaceae family, have recently been excluded from the *Enterobacter* genus following a taxonomy classification update. Most of these species were isolated from plants, but *Kosakonia cowanii* and *Kosakonia radicincitans* have rarely been reported as opportunistic human pathogens.

Our objective was therefore to evaluate whether the *mcr-kos* gene might encode resistance to polymyxins once expressed in clinically-relevant Enterobacterales and thus may possibly constitute a source of acquired resistance to these antibiotics. Genomic DNA from *K. sacchari* DSM 100203 was used for amplification of the *mcr-kos* gene. The minimum inhibitory concentration (MIC) of colistin for *K. sacchari* DSM 100203 was 4 mg/L. Susceptibility testing of β-lactams by disk diffusion according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (v.11.0) revealed a penicillinase phenotype of resistance, with resistance to amoxicillin and ticarcillin and slightly reduced susceptibility to piperacillin, all being reversed by the addition of clavulanic acid. In addition, the strain was resistant to fosfomycin and sulfonamides but remained susceptible to trimethoprim/sulfamethoxazole, aminoglycosides, quinolones, tetracycline and chloramphenicol.

The *mcr-kos* gene was cloned into plasmid pBAD and the recombinant plasmid was transformed both into *E. coli* TOP10 and wild-type *K. pneumoniae* CIP53153 reference strains. MICs of colistin were evaluated by broth microdilution as described previously [2] in the presence and absence of L-arabinose as inducer of gene expression for recombinant strains producing, respectively, MCR-KOS and MCR-1, that latter being used as a control [3]. *Escherichia coli* TOP10 producing MCR-KOS displayed a slight 2-fold increase (from 0.03 mg/L to 0.06 mg/L) in colistin MIC, whereas *E. coli* TOP10 producing MCR-1 displayed a 260-fold increase (from 0.03 mg/L to 8 mg/L). This result contrasts with the 8-fold increase in colistin MIC observed with the recombinant *E. coli* strain producing NMCR-2, a protein from *Kosakonia pseudosacchari* sharing 67% amino acid identity with MCR-8 and 92% with MCR-KOS [4].

Expression of the *mcr-kos* gene in *K. pneumoniae* also had a relatively low impact on the MIC of colistin, with a 4-fold increase (from 0.03 mg/L to 0.125 mg/L). Therefore, we aimed to evaluate whether MCR-KOS indeed acted as a potential EptA, as other MCR enzymes do. For that purpose, we analysed the lipid A moiety of the lipopolysaccharide (LPS) in *K. pneumoniae* CIP53153, which is the usual target of these enzymes leading to decreased susceptibility to polymyxins, using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) as described previously [5]. This showed that production of MCR-KOS in *K. pneumoniae* CIP53153 indeed generated a modification of the lipid A (additional peak at 1948 *m/z*), but at a different position compared with the other MCR enzymes in *K. pneumoniae* (peak at 1963 *m/z*). We might therefore speculate that the low increase observed in terms of colistin MIC could be linked to the addition of phosphoethanolamine in another position than position 4′ with other MCR enzymes.

Growth curves performed measured for recombinant *K. pneumoniae* strain CIP53153 (pBAD/*mcr-kos*) and its counterpart *K. pneumoniae* CIP53153 (pBAD) were compared but no significant difference was observed, thus showing that the high-level production of MCR-KOS did not impair the growth rate of the producing *K. pneumoniae* strain.

This report identified MCR-KOS, an EptA responsible for decreased susceptibility to colistin, whose gene exhibits significant

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identity with *mcr-8*, from the enterobacterial species *K. sacchari*. This further shows that environmental bacteria might represent sources of potential determinants leading to reduced susceptibility to colistin. This is another example of such a reservoir of MCR-like encoding genes, after *Moraxella pluranimalium* for MCR-2, *Aeromonas* spp. for MCR-3, and *Shewanella* spp. for MCR-4, all being environmental Gram-negative species. We may speculate that the fact that *mcr-kos* originates from an enterobacterial species makes the likelihood of further dissemination in other enterobacterial species more likely.

Although its impact on colistin susceptibility remained limited, it might anyhow contribute to reduced susceptibility to polymyxins if acquired by other bacterial species. The relatively low impact of MCR-KOS on the colistin MIC mirrors what has been previously shown with MCR-9, only increasing the MIC of colistin by 5-fold when overproduced in *E. coli* [5].

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