

already evolved to form Tn6172 (pHypo in Figure 1c). Using the *tni* genes and the IRL of Tn6022 together with the IRr of Tn6172, this segment has transposed as a single unit into the *comM* gene of the ancestor of the GC2 clone. Subsequently, AbGRI1 has evolved *in situ* into the large variety of forms seen in current GC2 isolates, some of which have acquired additional antibiotic resistance genes, such as *tet(B)*, *bla<sub>PER</sub>* or *oxa23* in Tn2006 or AbaR4.

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## Transparency declarations

None to declare.

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## MCR-2-mediated plasmid-borne polymyxin resistance most likely originates from *Moraxella pluranimalium*

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Sir,  
The recent identification of the first plasmid-mediated polymyxin resistance determinants, namely the MCR-1 and MCR-2 enzymes, has constituted an ultimate threat of pandrug resistance in Gram-negative organisms.<sup>1–3</sup> These enzymes have been reported only in Enterobacteriaceae, mainly in *Escherichia coli* and *Klebsiella*

*pneumoniae*; however, there have been reports from other enterobacterial genera, including *Enterobacter*, *Salmonella* and *Shigella*.<sup>1</sup> The *mcr-1* and *mcr-2* genes have both been identified from cattle and pigs.<sup>1–3</sup> In addition, the *mcr-1* gene has been identified from chickens,<sup>4</sup> but also from river samples and vegetables.<sup>5</sup>

MCR-1 and MCR-2, sharing 81% amino acid identity, are phosphoethanolamine transferases of 541 and 538 amino acids, respectively.<sup>2,3</sup> They add phosphoethanolamine to the lipid A moiety of LPS, leading to a more cationic LPS structure and consequently to resistance to polymyxins.<sup>1</sup>

We recently showed that some *Moraxella* species might constitute putative reservoirs of MCR-like proteins, with corresponding genes located on the chromosome of these species.<sup>6</sup> Hence, MCR-like proteins were identified in *Moraxella catarrhalis*, *Moraxella lincolni*, *Moraxella porci* and *Moraxella osloensis*. They all share significant amino acid identities with MCR-1 and MCR-2, ranging from 59% to 64%.<sup>6</sup> Even though these *Moraxella* species have been shown to carry intrinsic *mcr*-like genes, these genes remain quite distantly related to the plasmid-borne *mcr-1* and *mcr-2*.

We recently had the opportunity to investigate another *Moraxella* species. *Moraxella pluranimalium* strain 248-01<sup>†</sup> has been isolated from the nasal turbinate of a healthy pig in Spain.<sup>7</sup> *M. pluranimalium* is an aerobic and catalase- and oxidase-positive Gram-negative coccus that grows at temperatures of 22–37°C.<sup>7</sup>

PCR assays with internal primers specific for both *mcr-1* and *mcr-2*, as published,<sup>1</sup> allowed us to obtain an amplicon that was further sequenced. Internal outward primers were then designed and used for an inverse PCR strategy, as previously performed.<sup>6</sup> The sequence of the entire *mcr* gene was thus obtained and it revealed that this new enzyme (termed MCR-2.2) was almost identical to MCR-2 (99% amino acid identity), with only 8 amino acid differences out of the 538 constituting the MCR-2 enzyme, and shared 82% amino acid identity with MCR-1.

Interestingly, the *mcr-2.2* gene exhibits a G + C content of 49.1%, which is in accordance with the total G + C content of the genomes of different *Moraxella* species (~45%), which agrees with the intrinsic origin of this gene in *M. pluranimalium*.

The corresponding gene, named *mcr-2.2*, was cloned into plasmid pBADb, the recombinant plasmid being then expressed in *E. coli* TOP10 by adding L-arabinose 1% (necessary for the expression of the cloned genes in this inducible vector), as performed for other *mcr*-like genes.<sup>6</sup> Then MICs of colistin were determined for the recombinant strains expressing *mcr-1*, *mcr-2* and *mcr-2.2* by broth microdilution (BMD),<sup>1</sup> and this showed that MCR-2.2 conferred exactly the same level of resistance as MCR-1 and MCR-2 (MIC 4 mg/L compared with 0.03 mg/L for the *E. coli* WT recipient strain), thus showing that the few differences in terms of amino acid sequence did not have an impact on resistance to colistin in *E. coli*.

Interestingly, the MIC of colistin determined by BMD for *M. pluranimalium* strain 248-01 was high (16 mg/L). Susceptibility to other antibiotics, determined by disc diffusion, showed that this strain had a penicillinase phenotype, with resistance to amoxicillin and ticarcillin removed by clavulanic acid. In addition, it was resistant to tetracycline, nalidixic acid and ciprofloxacin, but remained susceptible to all aminoglycosides, sulphonamides, chloramphenicol and fosfomycin.

Since the *mcr-1* and *mcr-2* genes are often identified on IncX4-type plasmid scaffolds, strain 248-01 was tested for the corresponding replicase gene as reported,<sup>8</sup> but it remained negative. No plasmid could be identified despite several extraction attempts and attempts to transfer a polymyxin resistance determinant by electro-transformation into an *E. coli* recipient strain also failed.<sup>8</sup> These negative results further suggest a chromosomal location of the *mcr-2.2* gene. Since the *mcr-1* gene is most often associated with the IS*Apl1* element, which has been shown to be involved in its acquisition,<sup>9</sup> and since IS1595 was found to be closely associated with *mcr-2* (even though its involvement in the acquisition of the resistance gene has not yet been proven),<sup>3</sup> PCR specific for those IS elements was performed using DNA from strain 248-01<sup>†</sup> as a template. Interestingly, positive signals were obtained for both ISs and sequencing confirmed a perfect identity with IS*Apl1* and a variant of IS1595 (97% nucleotide identity), respectively. However, PCR mapping showed that these ISs were not located upstream of the *mcr-2.2* gene, in contrast to what is observed with both *mcr-1* and *mcr-2* genes when identified in enterobacterial isolates. This is another confirmation that IS*Apl1* may be found in *Moraxella* spp. after its identification in the genome of *M. porci*. Notably, the concomitant identification of IS1595 and of an *mcr-2*-like gene in the same *M. pluranimalium* strain further suggests that the mobilization event that led to the further dissemination of *mcr-2* in Enterobacteriaceae had first occurred from *M. pluranimalium*.

This report most likely identifies the direct progenitor of the *mcr-2* gene as *M. pluranimalium*, about which little is known so far. Strains belonging to this species have been recovered from pigs that were either healthy or suffering from pleuritis and polyserositis (nose, pleura and peritoneal cavity fluids), and from the brain of a sheep presenting with meningitis. It is likely that pigs might constitute a significant reservoir of *M. pluranimalium*. We speculate that the occurrence, selection, mobilization and dissemination of *mcr*-like genes might have occurred first in pigs. Since colistin is widely prescribed in veterinary medicine, in particular in pig farms, it represents a driving force for selection and spread of naturally occurring *mcr*-like resistance genes from *Moraxella* to Enterobacteriaceae. The present report paves the way for rethinking antibiotic policy in veterinary medicine, since this is likely a significant driving force for selection of antibiotic resistance traits that may have significant impacts in human medicine, as previously hypothesized.<sup>10</sup>

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## Transparency declarations

None to declare.

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## Identification of a novel *qnrA* allele, *qnrA8*, in environmental *Shewanella algae*

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Sir,  
Quinolones are recognized as one of the most widely prescribed classes of antibiotics used to treat infections caused by Gram-negative and Gram-positive bacteria.<sup>1</sup> In humans, quinolones are used to treat infections of the urogenital, respiratory and gastrointestinal tracts as well as a range of anatomically diverse infections in swine, poultry, cattle and companion animals and in aquaculture.<sup>2</sup> In the environment, fluoroquinolones break down slowly (half-life of ~100 days) and it is possible to measure trace levels of the drug in exposed environments.<sup>3</sup> The environmental impact of quinolones, particularly fluoroquinolones from humans, agriculture and pharmaceutical production facilities, is a cause of concern as residues and metabolic breakdown products released from the body of target species provides a selection pressure that impacts the ecology of non-target bacterial, invertebrate and vertebrate populations, where it can influence natural mutation rates and lateral gene transfer.<sup>2</sup>

The cellular targets of quinolones are bacterial type II topoisomerases, including DNA gyrase and topoisomerase IV.<sup>4</sup> DNA gyrase functions in the management of DNA supercoiling and topological stress, while topoisomerase IV has a role in unlinking replicated daughter chromosomes.<sup>4</sup> The genes encoding *QnrA*, *QnrB*, *QnrC*, *QnrD*, *QnrS* and *QnrVC* are found on the chromosome or on plasmids and confer resistance to quinolones and low-level resistance to fluoroquinolones. The *Qnr* proteins belong to the pentapeptide repeat family and function to protect DNA gyrase and topoisomerase IV, enabling bacteria to resist the actions of quinolones.<sup>5</sup>