Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of *Escherichia coli*

Acquired resistance to polymyxins is increasingly reported in Enterobacteriaceae, and particularly in *Klebsiella pneumoniae*. This increased resistance is extremely worrying considering that polymyxins are last-resort antibiotics for treating infections due to carbapenem-resistant Enterobacteriaceae.

Findings from a study by Yi-Yun Liu and colleagues[1] identified the plasmid-mediated *mcr-1* gene encoding a phosphoethanolamine transferase conferring resistance to polymyxins. This transmissible gene was identified from *Escherichia coli* and *K pneumoniae* isolates from animal sources (farm pigs, and retail meat from pork and chicken), but also from human sources, from several Chinese regions.[1]

We report here an *E coli* strain harbouring both plasmid-borne carbapenem and colistin resistance genes. That strain was recovered from urine cultures of an 83-year-old man who was admitted to hospital for diverticulitis in December, 2015, in Switzerland. He had renal deficiency and was therefore submitted to regular dialysis. *E coli* isolate KRI was resistant to most β-lactams (remaining susceptible to aztreonam) and resistant or of intermediate susceptibility to carbapenems (minimum inhibitory concentrations were 4 μg/mL for imipenem, 4 μg/mL for ertapenem, and 2 μg/mL for meropenem).[2] This isolate was also resistant to chloramphenicol, gentamicin, kanamycin, tobramycin, sulfonamides, tetracycline, co-trimoxazole, and fluoroquinolones, remaining susceptible only to amikacin, tigecycline, and fosfomycin. Noteworthy, it was resistant to colistin, with a minimum inhibitory concentration of 4 μg/mL.

PCR and sequencing revealed that *E coli* KRI harboured the bla_{VIM-1} carbapenemase[4] gene and the *mcr-1* gene. Additionally, the *floR* gene encoding resistance to florfenicol was identified. Mating-out assays done as previously described[3] identified the *mcr-1* gene on a roughly 60 kb plasmid, encoding resistance to colistin, chloramphenicol, florfenicol, and co-trimoxazole. Multilocus sequence typing identified *E coli* KRI as belonging to clonal complex (CC) 23.[4]

The origin of this *E coli* strain remains unknown since the patient did not travel abroad. An animal origin of the strains is indicated on the basis of the large usage of colistin in veterinary medicine that may have selected for colistin resistance, the florfenicol resistance trait widely observed with animal isolates related to florfenicol usage in veterinary medicine, and the recurrent identification of VIM-1-producing *E coli* strains in veterinary medicine. An animal origin of this strain reinforces the idea that antibiotic resistance issues should be considered as a one-health one-world approach. Finally, such accumulation of multidrug resistance traits may correspond to an ultimate step toward pandrug resistance in Enterobacteriaceae, considering that no new drugs against polymyxin resistant and metallo-β-lactamase producers will be marketed in the near future.

This work was financed by the University of Fribourg, Switzerland. We declare no competing interests.

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Published **Online** January 7, 2016 http://dx.doi.org/10.1016/S1473-3099(16)00006-2

Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids

Findings reported by Yi-Yun Liu and colleagues[1] identified the plasmid-borne gene *mcr-1* encoding resistance to colistin with a high prevalence in *Escherichia coli* isolates from animals, foodstuff, and human beings in China. The same gene was then reported in Europe (Denmark) among extended-spectrum β lactamase (ESBL) and AmpC-producing *E coli* isolates from chicken meat and human infections, but at a very low prevalence.[2]

We screened ESBL-positive *E coli* isolates collected in France for colistin resistance. Isolates were collected between 2005 and mid-2014 from faeces of diarrhoeic veal calves at farms, as part of a survey in the context of the French antimicrobial resistance Resapath surveillance network for animal pathogens. We screened these isolates for colistin resistance using disk diffusion and minimum inhibitory concentration determination by broth microdilution. We analysed plasmids bearing the *mcr-1* gene by conjugation, S1-pulsed-field gel electrophoresis, PCR-based replicon typing, and Southern blot. We analysed clonal relationship of all isolates by enterobacterial repetitive intergenic consensus PCR and pulsed-field gel electrophoresis.

Of 517 ESBL-producing *E coli* isolates collected, 106 (21%) were *mcr-1*

Published **Online** January 7, 2016 http://dx.doi.org/10.1016/S1473-3099(16)00007-4

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positive. Notably, the oldest *mcr-1*-positive *E coli* isolate had been collected in 2005. The 106 *mcr-1*-positive *E coli* isolates originated from different individuals located in 94 widely distant farms, and they were clonally unrelated.

Sequencing of the whole *mcr-1* gene in 75 *mcr-1*-positive isolates revealed a 100% identity compared with the original sequence. Co-occurrence of the *mcr-1* and *ESBL* genes was identified in a subset of seven isolates, with *mcr-1* and $bla_{CTX\text{-}M\text{-}1}$ being found on a large and conjugative IncHI2-type plasmid together with genes conferring resistance to sulfonamides and tetracyclines, two antibiotics widely used in veterinary medicine.

See Online for appendix

These findings demonstrate a colocation of the *mcr-1* gene along with an *ESBL* gene on a single plasmid, and additional studies are needed to clarify the diversity of the plasmid backbones spreading these two genes within our collection. Noticeably, the prevalence of the *mcr-1* gene among ESBL producers in veal calves was much higher than that found in ESBL-positive *E coli* isolates in human beings and chicken meat reported in Denmark.[2] This difference may reflect a major spread of the *mcr-1* gene in European live animals. We showed that the dissemination of *mcr-1*, at least in France, had already occurred more than a decade ago, with one *E coli* isolate collected in 2005 identified as *mcr-1* positive.

Altogether, available data reveal the occurrence of *mcr-1* among different animals and human contexts over time.[1–3] Worryingly, we show that selection pressure with broad-spectrum cephalosporins may select for colistin resistance and vice-versa, further highlighting the likelihood of a pandemic spread of *mcr-1*. Of note, the substantial use of tetracyclines and sulfonamides in animals might also substantially contribute to the dissemination of *mcr-1* plasmids.

In a one-health perspective, and considering the renewed importance of colistin in human medicine, our data and those from others underscore the urgent need to limit the spread of *mcr-1*-positive plasmids by reconsidering the massive use of colistin in veterinary medicine worldwide.

We declare no competing interests. This work was supported by the Agency for Food, Environmental and Occupational Health and Safety (ANSES), and by the University of Fribourg, Switzerland, and by a grant of the ANIWHA ERA-NET project (France/Switzerland). We thank all peripheral laboratories of the Resapath network, as well as Charlotte Petitjean for her technical help.

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Published Online January 7, 2016 http://dx.doi.org/10.1016/S1473-3099(16)00009-8

Colistin resistance gene *mcr-1* in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany

A plasmid-encoded gene conferring colistin resistance (*mcr-1*) was recently described by Yi-Yun Liu and colleagues[1] and subsequently reported in isolates from Denmark.[2] Unlike the previously described chromosomally encoded resistance mechanisms to colistin,[3] plasmid-encoded resistance can be transmitted by horizontal transfer from livestock, where colistin is used to treat infected animals, to human beings. Transfer of the resistance to multidrug resistant Enterobacteriaceae would seriously compromise current treatment options.

We searched for the presence of the *mcr-1* gene in our database of 577 whole genome sequences of isolates obtained from different sources (human, animal, and environmental) since 2009 in Germany (appendix). We detected the *mcr-1* gene in four *Escherichia coli* isolates, three originating from swine (R253, V163, 112065) and one from a human wound infection (NRZ14408). R253, V163, and 112065 are extended-spectrum β-lactamase-producing isolates that harbour $bla_{CTX\text{-}M\text{-}1}$, whereas the NRZ14408 human isolate carries a $bla_{KPC\text{-}2}$ carbapenemase gene in addition to *mcr-1* (appendix). The minimal inhibitory concentration of colistin in the isolates ranged from between 2 mg/L to greater than 16 mg/L. The genetic environment of *mcr-1* was variable, and not always associated with ISA*pl1* (figure). Colistin-resistance could be transferred by conjugation at rates of between 10^{-1} and 10^{-7} transconjugants per recipient. The *mcr-1* gene is located on conjugative IncHI2 plasmids in all isolates excepting V163, where it is present on an IncX4 plasmid.

The isolate V163 was obtained in 2010, indicating that the existence of transmissible colistin resistance in animal populations in Germany is not a recent occurrence. The detection of *mcr-1* on different classes of plasmids and their presence in isolates of various sequence types (appendix) suggests that multiple pathways for horizontal transmission of this resistance exist. Our data suggest that the advent of untreatable infections has already