



## Short Communication

## Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract *Enterobacteriaceae* in Switzerland



Nadia Liassine<sup>a</sup>, Laetitia Assouvie<sup>b,c</sup>, Marie-Christine Descombes<sup>d</sup>,  
Valérie Dénervaud Tendon<sup>b,c</sup>, Nicolas Kieffer<sup>b,c</sup>, Laurent Poirel<sup>b,c</sup>, Patrice Nordmann<sup>b,c,e,\*</sup>

<sup>a</sup> Dianalabs SA, Geneva, Switzerland

<sup>b</sup> Emerging Antibiotic Resistance, Medical and Molecular Microbiology Unit, Department of Medicine, University of Fribourg, Fribourg, Switzerland

<sup>c</sup> INSERM European Unit (LEA Paris), University of Fribourg, Fribourg, Switzerland

<sup>d</sup> Laboratoire Unilab, Coppet, Switzerland

<sup>e</sup> University of Lausanne and University Hospital Centre, Lausanne, Switzerland

## ARTICLE INFO

## Article history:

Received 21 July 2016

Received in revised form 9 August 2016

Accepted 11 August 2016

**Corresponding Editor:** Eskild Petersen,  
Aarhus, Denmark

## Keywords:

Polymyxins

MCR

Gram-negative

Transferable polymyxin resistance mediated by the *mcr-1* plasmid-borne gene has been described recently in *Enterobacteriaceae*, mostly in *Escherichia coli*, from animals, food, and patients in China.<sup>1</sup> This has raised considerable concern in view of pandrug resistance in *Enterobacteriaceae*. MCR-1-producing enterobacterial pathogens have been reported worldwide. Several cases of community- and hospital-acquired infection due to *mcr-1*-positive *E. coli* occurring in Switzerland (Geneva and Neuchâtel) in 2015 and 2016,<sup>2,3</sup> as well as MCR-1-producers in food and river water,<sup>4</sup> have been reported.

Very recently (July 2016), the MCR-2 plasmid-mediated colistin resistance determinant was identified in *E. coli* mainly from pigs in Belgium.<sup>5</sup> MCR-2, which is also a phosphoethanolamine transferase likely modifying the extremity of the lipopolysaccharide, shares 80.6% amino acid identity with MCR-1.

The aim of this study was to perform a prospective analysis of the prevalence rate of MCR-1- and MCR-2-producing *Enterobacteriaceae* in urine samples obtained from two main private laboratories in Switzerland during the period February to March 2016. The study was focused on urinary tract infections (UTIs) since (1) they still represent the main source of infection for humans, and (2) *E. coli* from community-acquired infections is at the interplay between the environment and hospitals, playing a potential role as an exchange platform for *mcr*-like genes. Strains were collected from the same region of Switzerland where *mcr-1*-positive *E. coli* was identified previously.

A total of 2049 non-duplicate enterobacterial isolates were screened. These included *E. coli* ( $n = 1704$ ), *Klebsiella pneumoniae* ( $n = 151$ ), *Proteus mirabilis* ( $n = 73$ ), *Citrobacter sp* ( $n = 32$ ), *Klebsiella oxytoca* ( $n = 22$ ), *Enterobacter cloacae* ( $n = 18$ ), *Morganella morganii* ( $n = 13$ ), *Enterobacter aerogenes* ( $n = 15$ ), *Proteus vulgaris* ( $n = 7$ ), *Serratia sp* ( $n = 7$ ), *Providencia rettgeri* ( $n = 3$ ), *Salmonella* group D ( $n = 2$ ), *Hafnia alvei* ( $n = 1$ ), and *Kluyvera ascorbata* ( $n = 1$ ). The predominance of *E. coli* isolates in this collection mirrors the

\* Corresponding author.

E-mail address: [patrice.nordmann@unifr.ch](mailto:patrice.nordmann@unifr.ch) (P. Nordmann).

known species distribution of pathogens of UTIs. The strains were first screened using the recently developed rapid polymyxin NP test, which is a rapid technique for the detection of colistin resistance.<sup>6</sup> Strains were then screened for the *mcr-1* and *mcr-2* genes using regular PCR techniques (*mcr-2* full Fw 5'-ATG ACA TCA CAT CAC TCT TGG-3' and *mcr-2* full Rv 5'-TTA CTG GAT AAA TGC CGC GC-3'; cycling conditions of 34 cycles of 95 °C × 1 min, 52 °C × 30 s, 72 °C × 1 min, followed by 1 cycle of 72 °C × 5 min) and the real-time PCR technique for the *mcr-1* gene.<sup>7,8</sup>

According to the results of the rapid polymyxin NP test, six isolates were resistant to colistin (prevalence rate, 0.29%): two *E. coli*, two *K. pneumoniae*, one *H. alvei*, and one *Salmonella* isolate. Determination of the minimum inhibitory concentration (MIC) performed by broth microdilution confirmed that these six strains were resistant to colistin (MIC > 2 mg/l), with MIC values of colistin ranging from 4 to >128 mg/l.

PCRs targeting the *mcr*-like genes performed on the colistin-resistant isolates remained negative. However, when testing colistin-susceptible isolates, a single *E. coli* strain (C1624) was positive for the *mcr-1* gene. The MIC value of colistin for this isolate was 0.125 mg/l (European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables for the interpretation of MICs and zone diameters, version 2.0, 2014). Southern hybridization using an *mcr-1* fragment of approximately 600 bp as a probe identified this gene located on a plasmid of approximately 35 kb. This plasmid was not transferable using liquid or solid mating techniques.<sup>7</sup> Multilocus sequence typing of *E. coli* C1624 showed that it belonged to sequence type ST428 (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/>).<sup>7</sup> This sequence type has been identified in infections of broiler breeders, further suggesting the animal origin of the MCR-like-producers.<sup>9</sup>

The overall prevalence rate of MCR-producing enterobacterial strains from human UTIs in this region of Western Europe remains very low (0.05%). This prevalence rate is somewhat similar to that observed recently in a large study of enterobacterial strains isolated worldwide from 2014 to 2015 (<0.1%).<sup>10</sup> It might be the consequence of a lack of selection of MCR-producing bacteria due to the lack of colistin use for the treatment of community-acquired infections. This low prevalence rate despite the use of colistin to treat infected animals in Switzerland suggests that a high transfer of *mcr*-like genes from animal (or environmental) strains to human strains has not already occurred. This further indicates the possibility of preventing the wide dissemination of *mcr*-like-producing bacteria in human medicine. However, the identification of a colistin-susceptible/*mcr-1*-positive isolate in the present study indicates that the silent spread of this gene might happen. An important step in preventing the spread of MCR-like-producers

would be to implement large and regular screening of animal isolates.

Finally, this study represents the first screening of human isolates for the *mcr-2* gene, a gene that has only been found in a few animal isolates. It is believed that this screening of MCR-producers characterizes the most recently available collection of strains. It may therefore represent the true prevalence rate of MCR-producers in this region of the world.

### Author contributions

Strain collection, NL, MCD; laboratory work, LA, VD, NK; study design, PN; writing, all authors.

**Funding:** This work was funded by the University of Fribourg and by a grant from the Office Fédéral de la Santé Publique, Switzerland.

**Ethical approval:** Not required.

**Conflict of interest:** The authors have no conflicts of interest to disclose.

### References

1. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2015;**16**:161–8.
2. Poirel L, Kieffer N, Liassine N, Thanh D, Nordmann P. Plasmid-mediated carbapenem and colistin resistance in a clinical isolate of *Escherichia coli*. *Lancet Infect Dis* 2016;**16**:281.
3. Nordmann P, Lienhard R, Kieffer N, Clerc O, Poirel L. Plasmid-mediated colistin-resistant *Escherichia coli* in bacteremia in Switzerland. *Clin Infect Dis* 2016;**62**:1322–3.
4. Zurfluh K, Poirel L, Nordmann P, Nuesch-Inderbinden M, Hächler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in ESBL-producing *Enterobacteriaceae* in river water and imported vegetable samples in Switzerland. *Antimicrob Agents Chemother* 2016;**60**:2594–5.
5. Xavier BB, Lammens C, Ruhel R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* 2016;**21**:27.
6. Nordmann P, Jayol A, Poirel L. Rapid detection of polymyxin resistance in *Enterobacteriaceae*. *Emerg Infect Dis* 2016;**22**:1038–43.
7. Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa. *Antimicrob Agents Chemother* 2016;**60**:4394–7.
8. Bontron S, Poirel L, Nordmann P. Real-time PCR for detection of plasmid-mediated polymyxin resistance (*mcr-1*) from cultured bacteria and stools. *J Antimicrob Chemother* 2016;**71**:2318–20.
9. Pires-dos-Santos T, Bisgaard M, Christensen H. Genetic diversity and virulence profiles of *Escherichia coli* causing salpingitis and peritonitis in broiler breeders. *Vet Microbiol* 2013;**162**:873–80.
10. Castanheira M, Griffin MA, Deshpande LM, Mendes RE, Jones RN, Flamm R. Detection of *mcr-1* among *Escherichia coli* clinical isolates collected worldwide as part of the SENTRY antimicrobial surveillance program during 2014–2015. *Antimicrob Agents Chemother* 2016. in press.