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Short Communication

Co-production of MCR-1 and NDM-1 by *Escherichia coli* sequence type 31 isolated from a newborn in Moscow, Russia

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ABSTRACT

An *Escherichia coli* sequence type 31 isolate co-harbouring *mcr-1* and bla_{NDM-1} genes on the plasmids of IncI2 and IncC groups, respectively, was recovered from a newborn with ventilator-associated pneumonia in Moscow, Russia. The convergence of polymyxin and carbapenem resistance and its expansion beyond Southeast Asia is a serious threat to human health.

Polymyxins are among the few effective antibiotics against carbapenemase-producing bacteria. The convergence of polymyxin and carbapenem resistance due to the co-production of *mcr*-type enzymes and carbapenemases is a serious health threat. It is still relatively rare worldwide; however, in China the prevalence among patients in the clinical setting is increasing (Huang et al., 2020). Here, we report the co-production of *mcr-1* and bla_{NDM-1} in a clinical *Escherichia coli* strain, sequence type 31 (ST31), outside the mentioned region.

A newborn in the city of Grozny (Chechen Republic, North Caucasus) was diagnosed with congenital heart disease. She spent the first 16 days of life in the neonatal intensive care unit (ICU), following which she was transferred to a hospital in Moscow, Russia, for surgery. Five days after the surgery, she was diagnosed with ventilator-associated pneumonia. *E. coli* isolate 5571 (Eco-5571) was recovered from a tracheal aspirate. The following minimum inhibitory concentrations were obtained through broth microdilution according to ISO standard 20776-1: cefepime >256 mg/l, meropenem 16 mg/l, ciprofloxacin 16 mg/l, cefotaxime 256 mg/l, gentamicin >256 mg/l, amikacin >256 mg/l, colistin 4 mg/l, tigecycline <0.12 mg/l, co-trimoxazole >128 mg/l, and fosfomycin 16 mg/l. The patient was successfully treated with meropenem and fosfomycin.

The Eco-5571 isolate was characterized using an Illumina MiSeq with Nextera XT 300-bp paired-end-reads library kit (Illumina Inc., San Diego, CA, USA). The reads were assembled using the SPAdes v.3.10.0 algorithm. In total, 139 contigs were assembled, amounting to 5,425,791 bp. The Center for Genomic Epidemiology services (http://www.genomicepidemiology.org/) were used for analysis (multilocus sequence typing, ResFinder, PlasmidFinder, and VirulenceFinder). Eco-5571 belonged to ST31 (Pasteur scheme). The following acquired antimicrobial resistance genes were detected: *aadA5*, *aadA2*, *aac(6')-Ib3*, *aac(3)-IId*, *rmtC*, $bla_{CTX-M-15}$, bla_{TEM-1b}, bla_{NDM-1}, bla_{CMY-6}, *mcr-1.1*, *mdf(A)*, *catA1*, *sul1*, *tet(A)*, *dfrA12*, and *dfrA17*. Furthermore, plasmid replicons IncC, IncFIB, IncFII, IncI1, and IncI2 were detected. The virulence genes were *air*, *cma*, *eilA*, *iron*, and *iss*.

PCR and Sanger sequencing revealed that bla_{NDM-1} was localized together with bla_{CMY-6} on a plasmid called pNDM-1_Msc_Eco-5571, which comprised a 128-kbp size scaffold from two contigs carrying an IncC replicon; however, its circularization was not confirmed (GenBank accession number MT119280). The *mcr*-carrying 59-kbp IncI2 plasmid, named pMCR-1_Msc-2, consisted of one contig, and its circularization was confirmed using PCR and Sanger sequencing; no other resistance genes were detected (GenBank accession number MT119279).

Plasmids demonstrating >90% nucleotide similarity and >90% coverage of scaffolds carrying bla_{NDM-1} and *mcr-1.1* were extracted from the GenBank database and subjected to cluster analysis using Prokka for plasmid annotation, Roary software for extracting the core genes, and FastTree (Price et al., 2010) for cluster analysis based on core-SNP alignment (Supplementary Material Figure S1). GenBank BLAST analysis of the bla_{NDM-1}-carrying scaffold revealed

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68 plasmids and a core genome of 57,099 bp. The closest plasmids were the following: pQD1501-Ct1 (132 kbp) obtained from a Chinese patient with *Klebsiella quasipneumoniae* nosocomial infection (Supplementary Material Figure S2) (GenBank accession number MN310375) (Cheng et al., 2019); unitig_1 (140 kbp) from a patient in the USA infected with *Klebsiella pneumoniae* (GenBank accession number CP018817.1); and pK71-77-1-NDM (145 kbp) from *E. coli* found in the blood of a Norwegian patient (GenBank accession number CP040884.1). The similarity analysis of the pMCR-1_Msc-2 plasmid sequence revealed 75 plasmids with a 22,424 bp core genome. The closest plasmids were pDR164 (59 kbp) obtained from an *E. coli* ST2280 isolate from a black kite (*Milvus migrans*) in Altay region (Tarabai et al., 2019) (GenBank accession number MK542639.1) (Supplementary Material Figure S3) and pSH16G4928 (277 kbp) obtained from *Salmonella enterica* serovar Typhimurium from a human isolate in China (GenBank accession number MH522426.1) (Lu et al., 2019).

Isolates similar to Eco-5571 had not been discovered at the Moscow hospital before the infant was admitted and none have been identified since. The infant had not had any contact with animals or the environment and had not been treated with colistin. NDM-type carbapenemase-producing *Enterobacteriaceae* are widespread in different regions of Russia, whereas *mcr*-type genes are rare (Ageevets et al., 2019). We have no data on the occurrence of *mcr-1* and $bla_{\text{NDM-1}}$ co-producing *E. coli* in the city of Grozny or in other regions of Russia.

China is one of the possible reservoirs of *mcr-1*-carrying Incl2 plasmids and of bla_{NDM}-carrying IncC plasmids, because the spread of both types of plasmid has been reported from this country (Elbediwi et al., 2019; Wu et al., 2019). Moreover a high proportion of *E. coli* (23%) co-harbouring both genes was detected in the poultry-producing chain in China (Wang et al., 2017). We can assume the existence of underestimated routes of long distance transmission of bla_{NDM} and *mcr-1* genes including international travel, the food trade, and even wild bird migration, which caused the importation of resistant isolates or particular genes from the probable reservoir in China to an ICU in Eastern Europe. However, we cannot completely exclude the local emergence of a strain carrying both genes. The One Health approach is required to understand the emergence and epidemiology of *mcr-1* and $bla_{\text{NDM-1}}$ co-producers.

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Ethical approval

Not required.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.09.1422.

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