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Letter to the Editor

Plasmid-mediated colistin resistance encoded by *mcr-1* gene in *Escherichia coli* co-carrying $bla_{CTX-M-15}$ and bla_{NDM-1} genes in pediatric patients in Qatar

Sir,

Colistin in combination with other agents is indicated as a last resort agent for the treatment of severe infections caused by multiple-drug resistant gram-negative bacteria, particularly those resistant to carbapenems. Worryingly, the recent emergence and global dissemination of the plasmid-mediated resistance gene *mcr-1*, which encodes a phosphoethanolamine transferase, threatens to render treatment with polymyxins ineffective [1]. This scenario has been further complicated by the presence in Enterobacteriaceae of incompatibility (Inc) type plasmids such as IncFII, IncX4, IncHI2A, and IncI2, able to carry genes encoding extended-spectrum beta-lactamases (ESBL) and carbapenemases together with *mcr-1* gene [1].

Although colistin resistance attributed to *mcr* genes have been reported in *E. coli* isolates recovered from broiler farms, poultry, and birds in the bird-market in the state of Qatar [2], these genes have seldom been found in human specimen [3]. An *E. coli* isolate MS8345 (ST95) having *mcr-1* borne on an IncHI2 plasmid was reported from a patient with a subarachnoid hemorrhage and was resistant to multiple antibiotics [3]. Herein, we report two multidrug-resistant *E. coli* strains carrying *mcr-1* gene along with bla_{CTX-M} and bla_{NDM} genes recovered from rectal swabs of pediatric patients during their routine admission screening for multidrug-resistant organisms to the Intensive Care Unit. Both isolates are part of our collection of ESBL-producing Enterobacteriaceae submitted for molecular typing and antimicrobial resistance genes characterization. The isolates have been inoculated onto two selective chromogenic media: CHROMagar™ ESBL and CHROMagar™ mSuperCARBA (CHROMagar, France) in the Microbiology laboratory and identified using the MALDI TOF Biotyper system (Bruker, Bremen, Germany). Antimicrobial susceptibility testing was performed using the BD Phoenix™ identification and susceptibility testing system (Becton Dickinson, USA). Minimum inhibitory concentrations (MIC) for colistin was determined by broth microdilution (ComASPTM Colistin, Liofilchem, Italy). MICs were interpreted according to the CLSI breakpoints. Whole genome sequencing was performed, and the sequence data was analyzed (For detailed information, see the supplementary text).

Colistin resistance in CP9 was attributed to the *mcr-1* located on a contig (27149 bp) that shared > 99% identity and > 98% coverage to many plasmids of different *E. coli* during BLAST search to NCBI databases; one of them was recognized as a IncI2 plasmid pBA76-MCR-1 (64942 bp, GenBank accession no. KX013540) harbored by a *mcr-1*-positive *E. coli* in Bahrain. The contig had the same architecture to pBA76-MCR-1 while *mcr-1* was located in between the relaxase and DNA topoisomerase III (Fig. 1). The isolate had also high-level resistance to carbapenems owing to the production of NDM-1 enzyme; annotation of the contig (6944 bp) carrying bla_{NDM-1} revealed the presence of insertional sequences IS*Aba14* and IS*Aba125* as well as bleomycin ble(MBL) located upstream and downstream of bla_{NDM-1} respectively [4]. The contig carrying bla_{NDM-1} also was co-localized with the aminoglycoside-modifying enzyme gene *aph(3′)-VI*. Genomics analysis identified other resistance genes conferring resistance to β-lactams ($bla_{CTX-M-15}$, bla_{TEM-1A}, bla_{OXA-9}), aminoglycosides (*aac(6′)-Ib*, *aadA1*, *aph(3′′)-Ib*, *aph(3′)-VI*, *aph(6)-Id*), sulfonamides/trimethoprim (*sul2* and *dfrA14*), and tetracyclines [*tet*(A)] (supplementary Table 1). Isolate CP9 belongs to ST115 and phylogenetic group A, which has been commensal and commonly detected in animals, livestock, poultry (43.3%) from Europe and United States and in five human cases from Denmark, Germany, Sri Lanka and Ecuador according to the MLST database (http://enterobase.warwick.ac.uk/species/index/ecoli).

The *mcr-1* gene in isolate E192 was found on a 61169 bp contig with an IncI2 replicon (revealed by Plasmidfinder); the contig was >99% identical to plasmids of at least five *E. coli* samples during BLAST search. Similar to the genetic organization of pk19EC149 (GenBank accession no. CP050290.1), the *mcr-1* gene was also located between relaxase and DNA topoisomerase III (Fig. 1). Analysis of the genome sequence confirmed the presence of multiple genes associated with resistance to β -lactams ($bla_{CTX-M-15}$, $bla_{TEM-141}$), aminoglycosides (*ant(2′′)-Ia, aadA1*), fosfomycin (*fosA3*), trimethoprim-sulfaamethoxazole (*sul1*, *sul2* and *dfrA1*), chloramphenicol (*floR*), and tetracyclines [*tet(A)*]. In addition, *E. coli* E192 displayed resistance to ciprofloxacin (MIC > 2 mg/L) owing to a single amino acid substitution in ParC topoisomerase IV (S80I) and double amino acid substitutions in DNA gyrase A (S83 L and D87 N) that are related to high-level fluoroquinolone resistance (supplementary Table 1). This isolate corresponds to ST540 and phylogenetic group D, which has been commonly reported in food and environmental sources (34%) according to the MLST database (http://enterobase.warwick.ac.uk/species/index/ecoli); isolates of human origin were also reported in various countries in Europe and the United States.

In terms of antibiotic stewardship, One Health Approach acknowledges human and animal health as one single interconnected unity in a particular environment. The presence of these two STs in animal and the environment could indicate the spread and dissemination of plasmid-mediated *mcr-1* from the environmental and animal sources to humans in local environment [1,5]. Independently of the source, the co-carriage of *mcr-1* and bla_{NDM-1} in *E. coli* clinical isolate possess an alarming challenge for antimicrobial stewardship programs in health care facilities in

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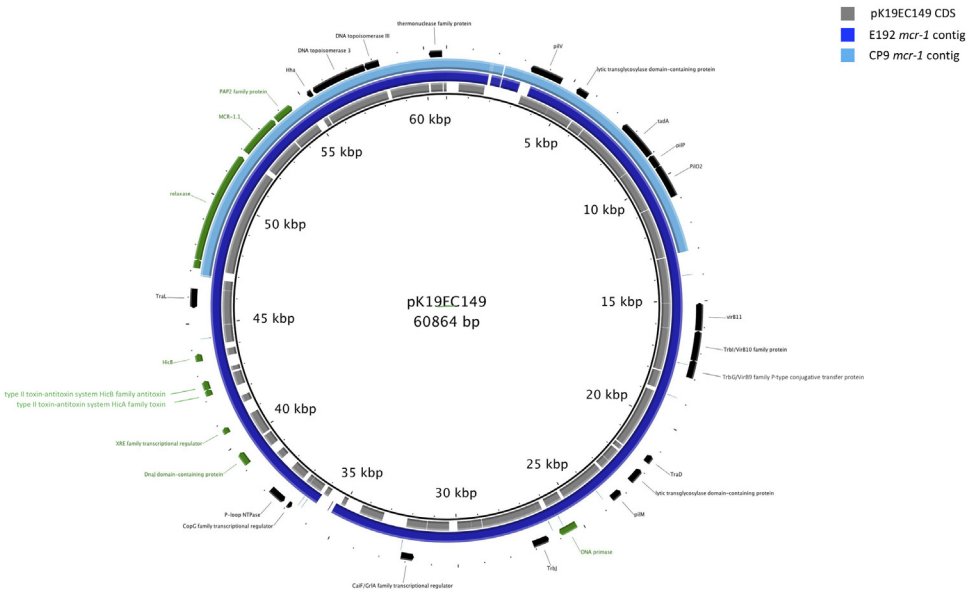

Fig. 1. The *mcr-1*–containing contigs from *E. coli* CP9 and E192 isolated from pediatric patients compared to pk19EC149 plasmid (CP050290.1). BRIG was used to generate a visual representation of the CP9 and E192 (27149 bp and 60864 bp contigs) assembled to pk19EC149. Coding regions of pk19EC149 are represented in the inner ring; the *mcr-1*–containing contigs are represented in the outer rings. Green and black arrows represent the major well annotated genes in the outer ring.

Qatar. Routine colistin testing for Enterobacteriaceae should be part of a coordinated approach to prevent the proliferation of bacteria harboring a plasmid with *mcr-1* and the carbapenemase.

Accession number: This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession PRJNA599369 as well as JABANQ000000000 and JABANR000000000. The version described in this paper is version JABANQ000000000 and JABANR000000000.

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Competing interests: none to declare.

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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.jgar.2020.06.029.

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