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OBSERVATION

First IncHI2 Plasmid Carrying *mcr-9.1*, bla_{VIM-1}, and Double Copies of bla_{KPC-3} in a Multidrug-Resistant *Escherichia coli* Human Isolate

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ABSTRACT We report a novel IncHI2 plasmid coharboring bla_{VIM-1}, two copies of bla_{KPC-3}, and *mcr-9.1* resistance genes in a human *Escherichia coli* isolate of the new serogroup O188. The bla_{VIM-1} gene was included in a class 1 integron, *mcr-9.1* in a cassette bracketed by IS*903* and ΔIS1R, and bla_{KPC-3} in two copies within a new composite Tn*4401*-like transposon. The emergence of carbapenem and colistin resistance genes in a single plasmid is of great concern for upcoming clinical therapies.

KEYWORDS colistin, carbapenems, multidrug resistance, IncHI2 plasmid, *Escherichia coli*

Carbapenems are considered antibiotics of choice against multidrug-resistant and extended-spectrum β-lactamase-producing strains, but the global increase of carbapenemase-producing *Enterobacteriaceae* (CPE) are compromising their use in therapy (1). Carbapenemases are frequently encoded by genes located on transferable elements and isolates of *Escherichia coli*, *Klebsiella*, and *Enterobacter* spp., carrying multiple carbapenemase-encoding genes on plasmids of different incompatibility (Inc) groups, have been reported (1). Colistin is often the last-line antibiotic against serious CPE infections; however, CPE strains with mobilized colistin resistance (*mcr*) determinants are emerging worldwide (2), further limiting the current therapeutic options.

In this study, we report the first human multidrug-resistant *E. coli* isolate (Ec3) coharboring bla_{VIM-1} and two copies of bla_{KPC-3} and *mcr-9.1* genes on the same IncHI2 plasmid. Ec3 was previously described as a highly resistant strain to imipenem, meropenem, and ertapenem (MICs of >128 μg/ml) but susceptible to colistin (MIC of 0.12 μg/ml) and tigecycline (MIC of 0.12 μg/ml), belonging to the sequence type ST1266 and PCR positive to bla_{VIM-1} and bla_{KPC-2} (3). S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) and following hybridization assays showed that bla_{VIM-1} and bla_{KPC-2} were located on a plasmid of ~250 kb. The unusual coharboring of two carbapenemase genes on the same plasmid prompted us to fully investigate the strain by whole-genome sequencing.

Genomic analysis performed using both the Illumina (Technological Platform Center of the University of Verona, Italy) and Oxford Nanopore DNA sequencing platforms (MicrobesNG, Birmingham, UK) revealed a genome consisting of 5.282,753 bp with a 50.5% GC content and the presence of a 249,437-bp plasmid with a 48% GC content. Sequencing data showed Ec3 strain belonged to E phylogroup and to O188:H34 serotype. Interestingly, the O188 serogroup, recently recognized in *E. coli*, shows a new O-antigen polysaccharide almost identical to *Shigella boydii* type 16 (4), suggesting the potential pathogenicity of Ec3 strain. In addition, some virulence genes (*pap*, *afaD*, and *chuA*) involved in adhesion and iron acquisition in diarrheagenic and

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uropathogen *E. coli* strains (5), were found by VirulenceFinder analysis on the Ec3 chromosome.

ResFinder analysis confirmed the strain carried multiple genes mediating resistance to beta-lactams (*ampC*, bla_{ACC-1}, bla_{OXA-1}, bla_{KPC-3}, and bla_{VIM-1}), aminoglycosides [*aadA1*, *aph(3'')-Ib*, and *aph(6)-Id*], fluoroquinolones [*aac(6')-Ib3*], sulfonamides (*sul1* and *sul2*), trimethoprim (*dfrA24*), chloramphenicol (*catA1*), tetracycline (*tetB*), and also to colistin (*mcr-9.1*), although the strain was phenotypically susceptible to polymyxins. Remarkably, the *mcr-9.1*, bla_{KPC-3}, and bla_{VIM-1} genes were located on the same plasmid (pEC3).

The pEC3 (GenBank accession no. MW509820) carried the IncHI2 replicon and belonged to the ST1 (6), showing the highest coverage (88%) and nucleotide identity (100%) with the VIM-1 and MCR-9 encoding plasmid pRH-R27 (GenBank accession no. LN555650) of *Salmonella enterica* isolated from a livestock farm in Germany (7). By BLASTN, pEC3 revealed similarity also to previously described IncHI2 plasmids carrying bla_{VIM} and *mcr-9* genes, like pME-1a (72% coverage and 99.97% identity, GenBank accession no. CP041734 [8]) and pMS37a (66% coverage and 99.97% identity, accession no. CP053191 [9]) from human or food *Enterobacter hormaechei* isolates, respectively (Fig. 1).

All beta-lactamase genes, except for *ampC* and bla_{OXA-1}, were located on pEC3. The bla_{ACC-1} gene, an AmpC-type beta-lactamase originated from *Hafnia alvei*, was associated with an IS*Ecp1* element as commonly reported for *Enterobacteriaceae* (10). The genetic context and sequence of bla_{ACC-1} were the same as in pRH-R27.

The bla_{KPC-3} gene, improperly reported as bla_{KPC-2} in the previous study (3), was bracketed by the IS*Kpn7* (upstream) and the IS*Kpn6* (downstream) within a Tn*4401a* transposon as first described by Naas et al. (11). However, Tn*4401a* was disrupted by the insertion into the *tnpR* gene of a further copy of the same truncated transposon in opposite direction (Fig. 2a). This arrangement was probably due to a recombination event between the two copies of Tn*4401a* transposon. The result was the loss of a 3,126-bp fragment and the formation of a composite transposon (of 16,688 bp) delimited by two identical IS (IS*Kpn6*) and containing two bla_{KPC-3} genes. The highest nucleotide similarity was observed with a portion of the IncF plasmid pECAZ147_KPC (accession no. CP018992) from a human *E. coli*. Adjacent copies of Tn*4401*-like transposons on the same plasmid have been reported in *Klebsiella pneumoniae* (12, 13), but their combination in a single transposable element has not yet been reported.

The bla_{VIM-1} gene was included in a class 1 integron almost identical to that carried by the plasmid pRH-R27 of *S. enterica*. The integron variable region contained the bla_{VIM-1}, *aacA4*, and *aadA1* gene cassettes. As in pRH-R27, the integron was in a Tn*21* transposon, also included in a Tn*9* homolog harboring the *catA1* gene for chloramphenicol resistance. However, in pEC3, a truncated *mer* operon downstream of the *tni* module of integron was observed (Fig. 2b).

The core structure of *mcr-9.1* cassette "*rcnR-rcnA-pcoE-pcoS*-IS*903-mcr-9.1-wbuC*" was identical to that described in other IncHI2 plasmids in different *Enterobacteriaceae* (14) and very similar to that of pRH-R27 (100% coverage and 99.96% identity). The regulatory genes (*qseC* and *qseB*), followed by IS*1R*, were detected downstream of the *wbuC* gene (Fig. 2c). Nevertheless, induction experiments, using subinhibitory concentrations of colistin (0.03 to 0.06 μg/ml), followed by quantitative real-time PCR (RT-qPCR) assays performed as described by Kieffer et al. (15), caused no increase of *mcr-9* gene expression and no higher MIC to colistin. The role of *qseC-qseB* on *mcr-9* induction may differ in isolates with different genetic backgrounds as suggested by Tyson et al. (16), but other genes may also be involved in the regulation of *mcr-9* expression (8, 14).

Since IncHI transfer rate is temperature dependent (17), conjugal experiments were performed at 37°C and 25°C, but they were both unsuccessful, consistent with deletions in one of the transfer regions. In particular, the *dsbC* and *traI* genes

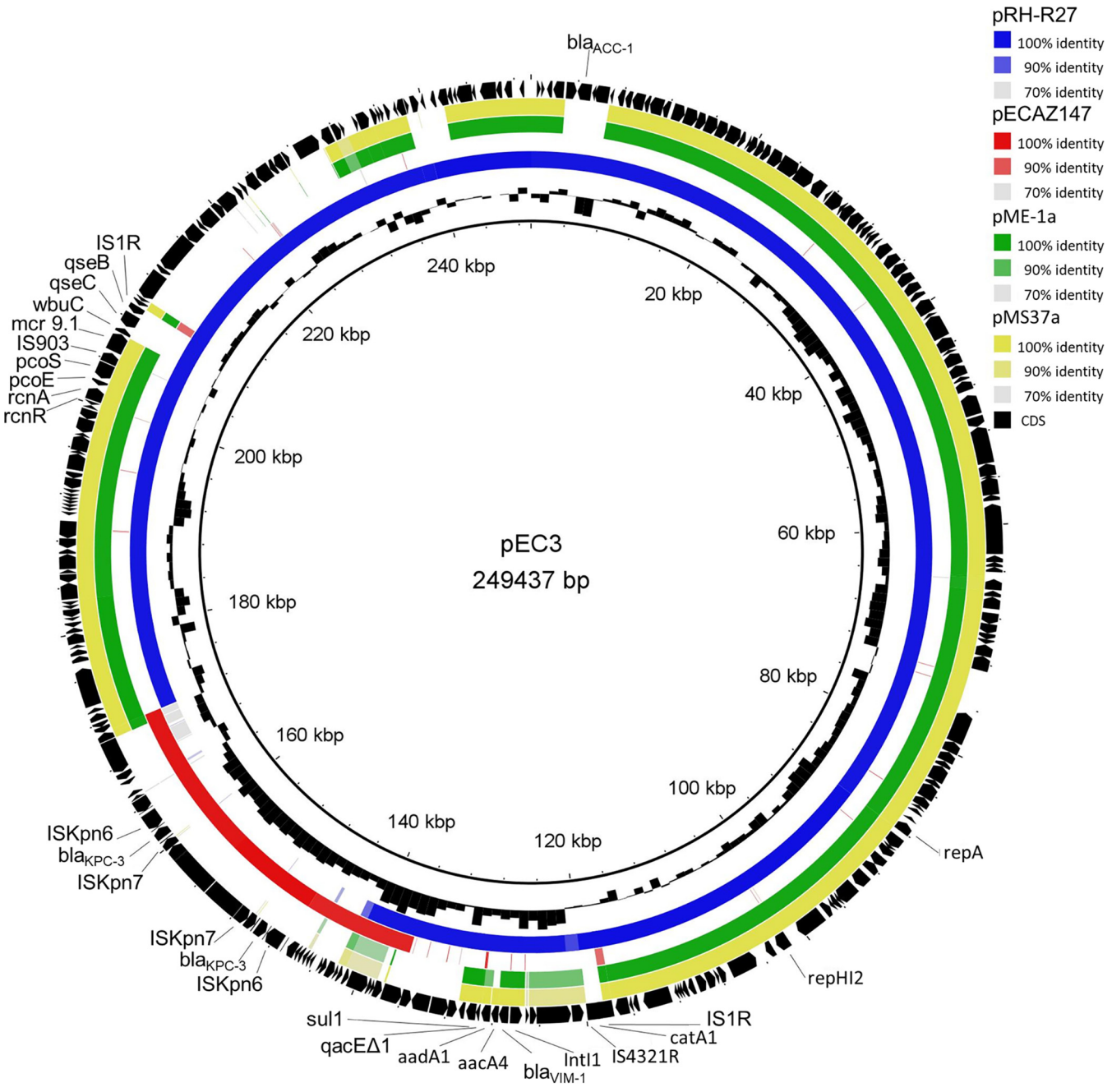

FIG 1 Genetic characterization of the IncHI2 plasmid pEC3. Circular map of the pEC3 plasmid coharboring *mcr-9.1*, bla_{VIM-1}, and bla_{KPC-3} in comparison with similar reported plasmids using BRIG software. The plasmids included in the analysis were as follows: (inner to outer circles) pRH-R27 (GenBank ID LN555650), pECAZ147_KPC (CP018992), pME-1a (CP041734), and pMS37a (CP053191). Black arrows indicate the positions and orientations of genes; some resistance and relevant genes described in this study are shown.

encoding a thioredoxin-like protein and relaxase, respectively, both involved in IncHI2 plasmid transfer, were not found in pEC3, as in a nonconjugative plasmid variant (pRH-R178) of pRH-R27 (7).

In conclusion, we describe in the new O188 *E. coli* serogroup, a novel IncHI2 plasmid coharboring *mcr-9.1*, bla_{VIM-1}, and bla_{KPC-3}. It likely originated by recombination with elements frequently associated with IncF plasmids and although nonconjugative, demonstrates that the ongoing spread of *mcr-9* and carbapenemase genes is caused by their association with genetic contexts able to move in different plasmids and bacteria.

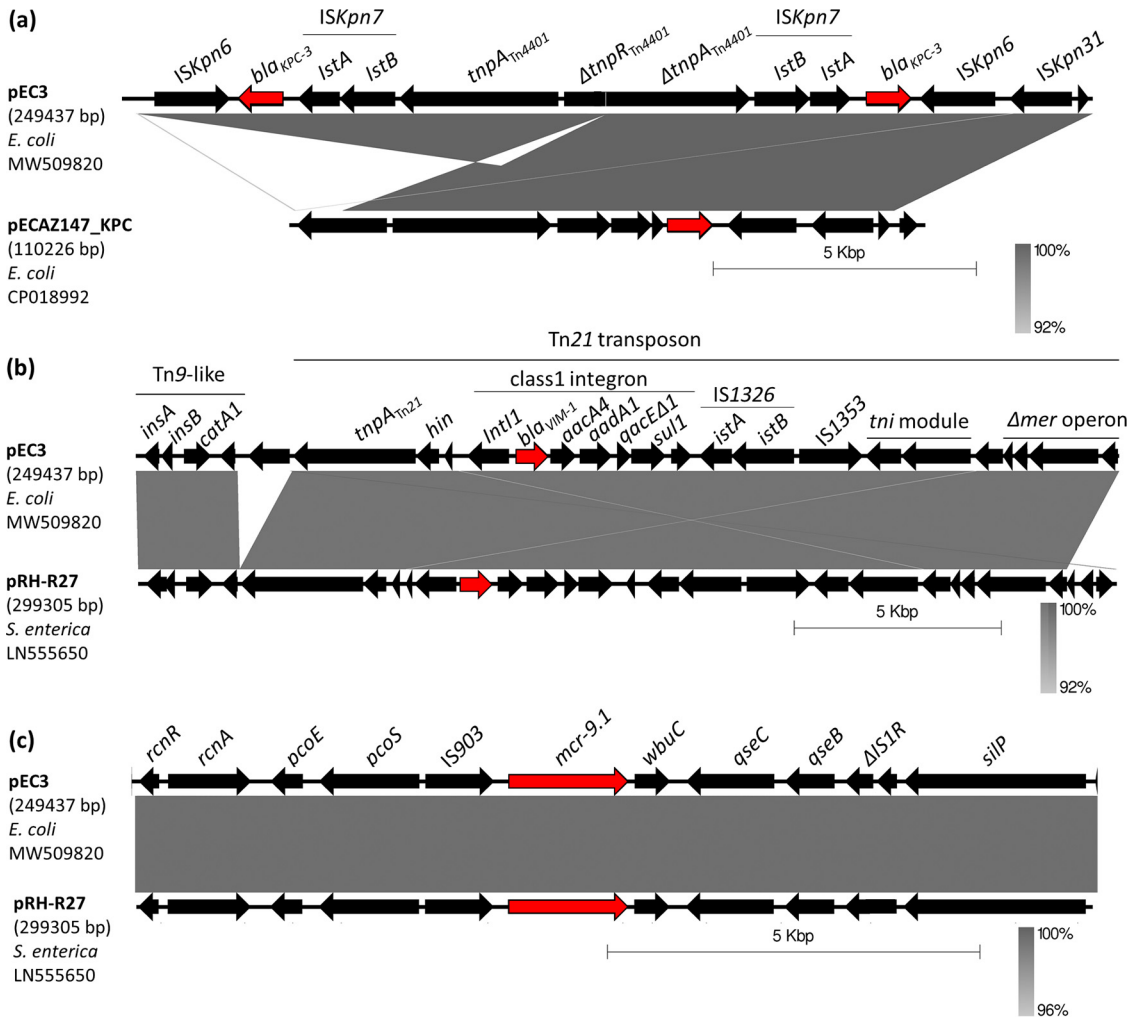

FIG 2 (a to c) Linear comparison of the bla_{KPC-3}, bla_{VIM-1}, and *mcr-9.1* contexts of pEC3 with the corresponding regions of highly similar plasmids pECAZ147_KPC and pRH-R27. Gray shading indicates regions of shared homology (ranging from 92 or 96% to 100%). The three resistance genes under study are shown by red arrows.

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