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MECHANISMS OF RESISTANCE

Spread of Clonally Related *Escherichia coli* Strains Harboring an IncA/C_1 Plasmid Encoding IMP-8 and Its Recruitment into an Unrelated MCR-1-Containing Isolate

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ABSTRACT Ten IMP-8-producing *Escherichia coli* isolates were recovered from surveillance cultures of a neonatal intensive care unit; eight of the isolates were clonally related. A 168.2-kb bla_{IMP-8} plasmid was fully sequenced, and it corresponded to the recently described IncA/C1-ST13 plasmid. This plasmid was detected in all isolates, even in those that were not clonally related. One unrelated isolate was also resistant to colistin and positive for *mcr-1*. This marker was located in a 62.7-kb IncI2 plasmid, which was also fully sequenced.

KEYWORDS *Escherichia coli*, IMP-8 metallo-beta-lactamase, IncA/C1 plasmid, IncI2 plasmid, *mcr-1*

The emergence and spread of carbapenemase-producing bacteria are major concerns for public health systems worldwide. IMP-type metallo-β-lactamases (MBLs) were first identified in the early 1990s in *Pseudomonas aeruginosa* in Japan and since then have been globally reported, mostly in *P. aeruginosa* and in other nonfermenting Gram-negative bacilli (1). Studies performed in Argentina reported the presence of IMP-13 in *P. aeruginosa* and IMP-8 in *Enterobacter cloacae* (2–4). IMP-8 was initially described in *Klebsiella pneumoniae* in Taiwan, where it became the dominant MBL among *Enterobacteriaceae* (5). The presence of the bla_{IMP-8} gene was reported in nosocomial and environmental *E. coli* isolates in association with conjugative plasmids belonging to IncA/C and IncFIB, respectively. As in other MBL-coding genes, bla_{IMP-8} was found to be located in a class 1 integron (4–6).

The rising frequency of carbapenem-resistant *Enterobacteriaceae* infections prompted the use of colistin as a last therapeutic option. However, the scenario became more complex as a consequence of the silent spread of plasmid-carried *mcr-1* in the past decade (7, 8).

The aim of this study was to characterize carbapenem-resistant *E. coli* isolates recovered from the active surveillance cultures of a neonatal intensive care unit (NICU) at a hospital in Buenos Aires, Argentina. Surveillance cultures are routinely conducted on patients admitted to the NICU in this hospital. During November to December 2016, 10 carbapenem-resistant *E. coli* isolates were recovered from seven patients on CHROMagar KPC (*K. pneumoniae* carbapenemase). Antibiotic susceptibility was assessed by microdilution tests according to the CLSI guidelines and the use of automated systems (Vitek 2, bioMérieux, France). All isolates were found to be resistant to trimethoprim-sulfamethoxazole and cephalosporins, with intermediate susceptibility or resistance to imipenem and/or meropenem (9). Nine of the 10 isolates were resistant to amikacin, gentamicin, and ciprofloxacin, and they displayed a wild-type phenotype

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with respect to colistin (i.e., they were susceptible according the EUCAST guidelines). The one remaining isolate was categorized as non-wild type for colistin (i.e., resistant according the EUCAST guidelines), and it was intermediate for aminoglycosides and ciprofloxacin (Table 1) (9). All isolates showed a positive synergy test result with EDTA (1 μmol), suggesting the presence of an MBL (10, 11). PCR amplifications for the most common MBL-coding genes were conducted with specific primers and plasmid DNA as the template (11–13). Nucleotidic sequences of the amplified fragments showed 100% identity with bla_{IMP-8} for all samples. This marker was located on a conjugative plasmid, which was successfully transferred to *E. coli* CAG 12177. According to a PCR-based replicon typing method proposed previously, the plasmid corresponded to the IncA/C group, similarly to those seen in isolates previously reported in Singapore (5, 14).

bla_{IMP-8} was located in a 168.2-kb plasmid, which was purified by the standard plasmid DNA phenol-chloroform purification protocol initially proposed by Kado and Liu, adding two extraction steps performed with chloroform to remove any remaining phenol (15). Purified plasmids were fully sequenced by the use of a MiSeq sequencer (Illumina). The sequencing reads were assembled using SPAdes V3.9 with the following statistical parameters: largest contig, 153,183 bp; N50, 90,320 bp; L50, 2. The plasmid presented 227 open reading frames and had an average of 50.3% G+C content. Genes were predicted and annotated using the RAST tool and PROKKA software and were also manually curated (GenBank accession no. [AN] MG550958) (16). Using the ResFinder tool, other resistance markers such as bla_{TEM-1B}, *aadA1*, *aph(3′)VIa*, and *sul1* were detected in the same plasmid. bla_{IMP-8} was associated with a class 1 integron flanked by two IS*26* elements. The bla_{IMP-8}-carrying integron lacked the typical 3′ conserved sequence (*qacEΔ1* and *sul1*) but instead harbored a truncated sequence of a retron-type RNA-directed DNA polymerase (maturase), which has been reported to be likely involved in cassette gene generation (17, 18) (Fig. 1). The genetic platform for bla_{IMP-8} was confirmed in all isolates by PCR mapping and sequencing performed with the primers shown in Fig. 1. Plasmid multilocus sequence typing (pMLST) was performed to determine the IncA/C replicon type (https://pubmlst.org/plasmid/). The IncA/C_1 replicon, detected in this plasmid, was coincident with data corresponding to the recently deposited sequence type 13 (ST13) plasmid from *Citrobacter freundii*, which was isolated 20 years ago in Argentina. These plasmids are closely related to the ST11 IncA/C_1 RA1 plasmid and to the recently published ST12 (19, 20).

As previously mentioned, one isolate (*E. coli* G3216) was also colistin resistant and rendered a positive PCR result for *mcr-1*; this gene was located in a conjugative plasmid that was successfully transferred to *E. coli* CAG 12177. In accordance with previous reports, the full sequence of the *mcr-1*-harboring plasmid (pG3216) showed that *mcr-1* was flanked upstream by *pap2* (type 2 phosphatidic acid phosphatase) and downstream by the relaxase NikB-coding gene (GenBank AN MF693349). This plasmid was 62.7 kb in size and presented 88 open reading frames with an average of 42.5% G+C content. It did not harbor any further resistance gene and was almost identical (99% identity) to that previously reported in our country (GenBank AN KY471314) (21). Using the PlasmidFinder tool, pG3216 was found to be associated with the IncI2 group. Hence, *hicA* and *hicB*, which are related to the IncI2 incompatibility group and involved in type II toxin-antitoxin (TA) systems, were identified (22). The coding genes for the toxin RelE and the antitoxin StbE, which belong to the RelE/ParE TA system superfamily, were located together (23). Similarly to other IncI2 plasmids, pG3216 displayed a typical backbone responsible for plasmid replication, maintenance, and self-transfer by conjugation (24).

The *E. coli* phylogenetic group was determined according to the method previously described by Clermont et al. (25), and all of the isolates corresponded to phylogroup D (25). The clonal relationship was investigated by the use of XbaI pulsed-field gel electrophoresis (XbaI-PFGE) and MLST (http://enterobase.warwick.ac.uk/species/ecoli/allele_st_search). Eight of the 10 isolates were clustered in pulsotype I and did not correspond to any of the assigned sequence types (*adk* 332, *fumC* 594, *gyrB* 428, *icd* 517, *mdh* 292, *purA* 373, *recA* 262). *E. coli* G1216 presented a different pulsotype

TABLE 1 Clinical data and microbiological characteristics of bla_{IMP-8}-harboring isolates[a]

Newborn	Bacterial isolate/day of isolation[b]/no. of days of NICU stay before isolation	Treatment/no. of days of treatment	Risk factor/GA/wt of newborn (g)	Antimicrobial susceptibility (MIC [μg/ml])[c]													Pulsotype
				AM	AMS	PTZ	FEP	CAZ	CTX	IMP	MEM	GEN	AKN	CIP	COL	TMS	
1	G2116/1/89	MEM/21, COL/21, VAN/21	Preterm birth/30 wks/870	>32	>32	>128	>64	>64	>64	8	16	>16	>64	1	2	>320	I
2	G1116/13/40	AM/10, GEN/7, CTX/5, AKN/5, COL/8	Preterm birth/28 wks/1,260	>32	>32	>128	16	>64	>64	4	8	>16	>64	1	1	>320	I
	G1216/28/56			>32	>32	>128	>64	>64	>64	8	8	>16	>64	1	1	>320	II
	G1316/41/69			>32	>32	>128	>64	>64	>64	2	8	>16	>64	1	1	>320	I
3	G4116/28/41	AM/10, GEN/7	Term birth/40 wks/3,370	>32	>32	>128	>64	>64	>64	8	16	>16	>64	1	1	>320	I
4	G3116/28/8	CTX/4, AKN/4	Term birth/38 wks/3,210	>32	>32	>128	>64	>64	>64	8	8	>16	>64	1	2	>320	I
	G3216/41/21			**>32**	**>32**	**>128**	**>64**	**>64**	**>64**	**4**	**8**	**8**	**32**	**<0.25**	**8**	**>320**	**III**
5	G6116/43/16	AM/5, GEN/5	Preterm birth/34 wks/2,060	>32	>32	>128	>64	>64	>64	2	8	>16	>64	1	1	>320	I
6	G7116/43/28	VAN/12, MEM/12	Preterm birth/32 wks/1,610	>32	>32	128	>64	>64	>64	2	8	>16	>64	1	1	>320	I
7	G5116/49/41		Preterm birth/37 wks/2,500	>32	>32	>128	>64	>64	>64	2	8	>16	>64	1	1	>320	I

[a]AM, ampicillin; AMS, ampicillin-sulbactam; PTZ, piperacillin-tazobactam; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; IMP, imipenem; MEM, meropenem; GEN, gentamicin; AKN, amikacin; CIP, ciprofloxacin; COL, colistin; TMS, trimethoprim-sulfamethoxazole; VAN, vancomycin; GA, gestational age.

[b]For the data representing the day of isolation, day 1 corresponds to the index case; the other days of isolation were determined with respect to that of the index case.

[c]MICs for IMP, MEM, and COL were assessed by manual procedures, whereas those of the others were assessed by automated methods (Vitek 2). Data corresponding to the *mcr-1*-harboring isolate are indicated in bold.

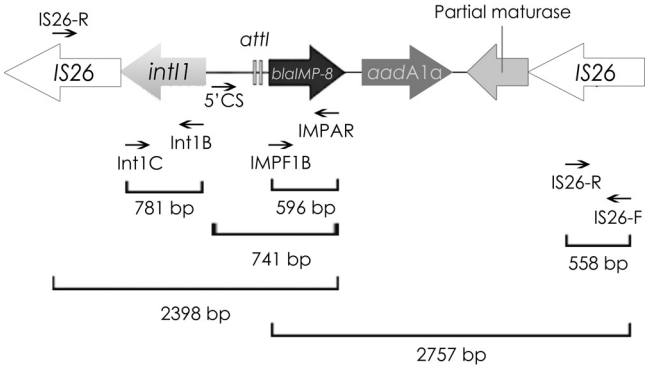

FIG 1 Genetic context of plasmid-borne bla_{IMP-8} detected in this study (MG550958). The primers used for PCR mapping were as follows: IMP-F1B (GTTTTGTAGCATTGCTACCGCAG) and IMP-AR (GTTTTGCCTTACC ATATTTGGA), IS26-F (TCACTCCACGATTTACCGCT) and IS26-R (CTTACCAGGCGCATTTCGCC), Int1B (GCGT TCGGTCAAGGTTCTGG) and Int1C (CGTGATGCCTGCTTGTTCTA), and 5′CS (GCTTGCTGCTTGGATGCC).

(pulsotype II), which corresponded to, among others, a single-locus variant (SLV) of ST69 (CC69) (*adk* 21, *fumC* 35, *gyrB* 27, *icd* 6, *mdh* 286, *purA* 5, *recA* 4). The *mcr-1*-positive *E. coli* G3216 isolate represented pulsotype III, which corresponds to a SLV of ST5377 and ST7395 (*adk* 35, *fumC* 37, *gyrB* 29, *icd* 25, *mdh* 416, *purA* 564, *recA* 73). These results indicate that the isolates included in this study do not belong to the STs in which bla_{IMP-8} was previously reported (ST131, ST359, ST457, and ST410) (5, 26).

Despite the fact that IMP-8-producing *Enterobacteriaceae* are frequently detected in Asia, in our country (Argentina), they have been encountered only sporadically (4). Both CHROMagar KPC and EDTA-based synergy tests were useful to perform early and accurate detection of colonized neonates with MBL-producing *E. coli*, thereby contributing to the reduction of the spread of such microorganisms and probably of the subsequent infections. *E. coli* phylogroup D includes extraintestinal pathogens and multidrug-resistant isolates (27). Although these strains are reported to be responsible for severe diseases, none of the seven neonates included in this study developed IMP-8 *E. coli* infections. Moreover, none of them received antibiotic treatment for this colonization and all of them were medically discharged. Dissemination of *E. coli* which produced IMP-8 MBL and belonged to pulsotype I occurred in this neonatal ward until hygiene measures and contact isolation were strengthened. It can be speculated that horizontal transmission of the bla_{IMP-8} Inc A/C_1 plasmid may have occurred, as this marker was also detected in the unrelated isolates; *E. coli* G1216 (pulsotype II) recovered from a patient also colonized with pulsotype I *E. coli* and *E. coli* G3216 (pulsotype III), which also harbored the IncI2 plasmid containing *mcr-1* (Table 1). Furthermore, the class 1 integron platform containing bla_{IMP-8} could be self-mobilized as it was flanked by two identical IS*26* copies, which could act as composite transposons (28).

Plasmid-mediated colistin resistance, commonly found in carbapenem-susceptible strains, has also been reported in *Enterobacteriaceae* producing different carbapenemases such as VIM-1, VIM-2, NDM-5, NDM-9, IMP-4, KPC-2, and OXA-48 (29–35). In our country, *mcr-1* was previously described both in clinical isolates and in poultry farms (8, 36). Here, we have described the presence of *mcr-1* in a bla_{IMP-8}-producing *E. coli* isolate, which adds an extra level of plasticity in the evolving epidemiology of carbapenem and colistin resistance.

Our results highlight the importance of establishing screening schemes and rapid laboratory diagnostic tests to ensure the implementation of efficient infection control measures.

Accession number(s). The complete sequences of the plasmids analyzed in this study have been deposited at DDBJ/EMBL/GenBank under accession numbers MG550958 and MF693349.

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