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Unusual association of NDM-1 with KPC-2 and *arm*A among Brazilian Enterobacteriaceae isolates

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Abstract

We report the microbiological characterization of four New Delhi metallo-β-lactamase-1 (bla_{NDM-1})-producing *Enterobacteriaceae* isolated in Rio de Janeiro, Brazil. bla_{NDM-1} was located on a conjugative plasmid and was associated with *Klebsiella pneumoniae* carbapenemase-2 (bla_{KPC-2}) or aminoglycoside-resistance methylase (*arm*A), a 16S rRNA methylase not previously reported in Brazil, in two distinct strains of *Enterobacter cloacae.* Our results suggested that the introduction of bla_{NDM-1} in Brazil has been accompanied by rapid spread, since our isolates showed no genetic relationship.

Key words: *Enterobacteriaceae*; Carbapenem-resistance; Carbapenemases; Methylase

Introduction

Antimicrobial resistance is a growing global challenge to human health. The New Delhi metallo-β-lactamase (NDM), an acquired class B carbapenemase, has gained public attention due to its rapid worldwide dissemination. bla_{NDM-1} was first identified in *Klebsiella pneumoniae* and *Escherichia coli* from India and Pakistan in 2008 (1,2). Subsequent studies reported NDM-1 from hospitals located in other Indian cities, as well as from drinking water and seepage water in New Delhi (3). To date, NDM-1-producing isolates have been reported in more than 50 countries (1,4). While many of the patients carrying NDM-1-producing strains had a history of hospitalization in India, Pakistan or Bangladesh, others had simply travelled to this region, which may indicate community acquisition of NDM-producing bacteria by ingestion of contaminated water and/or food. In Brazil, the first report of NDM-1 occurred in July 2013 in a *Providencia rettgeri* strain isolated from Rio Grande do Sul (5). In this study, we characterized four *Enterobacteriaceae* clinical isolates that were NDM-1 producing and were recovered at different hospitals in Rio de Janeiro, Brazil.

Material and Methods

Four carbapenem-resistant *Enterobacteriaceae* strains were isolated from various sites from four different patients admitted at four different hospitals located in two cities in the state of Rio de Janeiro between September and October 2013. These isolates were identified as *P. rettgeri* and *Enterobacter cloacae* (ECL) (n = 3) using a Vitek® 2 Compact automated system (bioMérieux Clinical Diagnostics, France). These isolates were resistant to carbapenems, with a minimum inhibitory concentration (MIC) ⩾2 μg/mL for imipenem or meropenem and an MIC ⩾1 μg/mL for ertapenem. Because of that, they were screened for carbapenemase production by testing commercially available disks containing carbapenems with and without EDTA (0.1 M), cloxacillin (75 mg/mL) or phenylboronic acid (40 mg/mL), as recommended by the National Health Surveillance Agency (ANVISA) technical guidelines (6).

Since the diameter-zone difference between the carbapenem/EDTA and carbapenem disks was ⩾5 mm, these strains were screened as possible metallo-β-lactamase (MBL) producers and referred to the Laboratório Especial de Microbiologia Clínica, UNIFESP, São Paulo, SP, Brazil for further molecular characterization. The *Enterobacteriaceae* strains were re-identified at the species level by matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) mass spectroscopy (Bruker Daltonics, Germany), using the Biotyper 3.0 software (Bruker Daltonics, Germany), and 16S rRNA DNA sequencing.

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The antimicrobial susceptibility profile was performed and interpreted by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution guidelines (7), except for polymyxin B, for which the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (8) breakpoints were applied. The MBL phenotype was investigated by the MBL imipenem/imipenem-inhibitor (IP/IPI) E-test method (AB Biodisk, Sweden). Detection of the following encoding genes, bla_{NDM}, bla_{KPC}, bla_{GES}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{VIM}, bla_{CTX-M}, bla_{SHV}, bla_{TEM}, bla_{OXA-48}, and 16S rRNA methylase genes, *rmt*A, *rmt*B, *rmt*C, *rmt*D, *rmt*G, *arm*A and *npm*A, was carried out by PCR followed by DNA sequencing (ABI sequencer, Applied Biosystems, USA).

Clonal relatedness among ECL isolates was evaluated by pulsed field gel electrophoresis (PFGE) using the SpeI restriction endonuclease (New England BioLabs, USA). The band patterns were analyzed by visual inspection, applying the criteria established by Tenover et al. (9). Conjugation and transformation experiments were performed using *E. coli* J53 ($LacZ^-$ Nal^r Rif^r) and *E. coli* DH5α, respectively, as recipient strains. Transconjugants were selected on MacConkey agar, using imipenem (0.5 μg/mL) and azide (150 μg/mL). Plasmids and whole genome DNA were extracted from the strains and from *E. coli* J53 by the Kieser method (10) and with a QIAamp DNA Mini Kit (Qiagen, Germany), respectively, and analyzed using a 0.8% agarose gel. A replicon-based PCR typing of plasmid incompatibility groups (Inc/rep) was performed using previously published protocols (11,12) with 5 multiplex and 6 single reactions recognizing the FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, FIIA, and Q replicons.

Results

The results of this study are summarized in Table 1. The initial identification of bacterial species was confirmed, as well as the MBL phenotype, by the E-test. All

Table 1. Molecular and phenotypic characterization of NDM-1-producing isolates in Rio de Janeiro, Brazil.

Strains	*P. rettgeri* E132	*E. coli* J53 + pECLE132	*E. cloacae* E133	*E. cloacae* E134	*E. coli* J53 + pECLE134	*E. cloacae* E135	*E. coli* J53
Site of isolation	Catheter	–	Urine	Blood	–	Ascitic fluid	–
PFGE pattern	–	–	A	B	–	C	–
MIC (μg/mL)*							
IMI	32	8	16	16	32	8	0.06
IMI/AC	32	8	16	16	16	8	–
Mero	32	8	32	12	64	4	0.13
MERO/AC	32	8	32	16	16	32	–
Erta	256	64	128	256	128	16	0.01
Erta/AC	128	32	256	256	128	128	–
Poly B	>128	<0.125	0.25	0.25	<0.25	<0.25	<0.25
CEF	256	128	512	>512	256	64	0.06
Cipro	>256	0.016	>32	64	0.016	0.032	0.008
Levo	>256	<0.25	64	16	<0.25	<0.25	0.125
CAZ	>512	>512	>512	>512	>512	>512	0.125
Tige	0.5	0.06	0.25	1	0.06	0.25	0.25
CRO	512	512	>512	>512	>512	512	–
CFL	>512	>512	>512	>512	>512	>512	–
Genta	0.25	2	>128	64	1	1	0.125
Amica	4	2	>256	4	2	4	<2
CTX	>512	>512	>512	>512	>512	>512	0.06
MBL E-test	+	+	+	+	+	+	–
Inc/rep typing	HI2 and P	P	HI2 and W	P and N	P	**	–
bla genes	NDM-1 and TEM-1	NDM-1	NDM-1, TEM-1, CTX-M-15 and armA	NDM-1, KPC-2, TEM-1 and CTX-M-15	NDM-1	NDM-1, TEM-1 and CTX-M-15	

NDM-1: New Delhi metallo-β-lactamase-1; PFGE: pulsed field gel electrophoresis; IMI: imipenem; AC: clavulanic acid; Mero: meropenem; Erta: ertapenem; Poly B: polymixyn B; CEF: cefepime; Cipro: ciprofloxacin; Levo: levofloxacin; CAZ: ceftazidime; Tige: tigecycline; CRO: ceftriaxone; CFL: cephalothin; Genta: gentamicin; Amica: amikacin; CTX: cefotaxime; MBL: metallo-β-lactamase.
* Minimum inhibitory concentrations (MICs) were determined by agar dilution [Clinical and Laboratory Standards Institute (CLSI) 2013].
** The E135 sample tested negative for all plasmid incompatibility groups.

isolates were resistant to broad-spectrum cephalosporins, ertapenem, imipenem, and meropenem. The ECL isolates were susceptible to polymyxin B, but the *P. rettgeri* isolate (#E132) was resistant to this agent, as expected. The presence of bla_{NDM-1} *and* bla_{TEM-1} was confirmed in all isolates. All ECL isolates carried $bla_{CTX-M-15}$ and possessed different PFGE patterns. Co-occurrence of bla_{NDM-1} with *arm*A or with bla_{KPC-2} genes was detected in two distinct ECL strains, E134 and E133, respectively. The transference of bla_{NDM-1} to a recipient strain was achieved for strains E132 and E134, suggesting that this gene was probably located on a ~230 kb conjugative plasmid that belonged to the Inc/rep P type. The transference of bla_{NDM-1} by conjugation failed for the remaining isolates. Hybridization revealed that the bla_{KPC-2} gene was located on the chromosome of the E134 isolate. This finding explained why repetitive attempts to transfer bla_{KPC-2} by conjugation have failed. Electroporation experiments were unsuccessful. Transconjugant strains showed the same elevation of carbapenem MICs as exhibited by donor strains.

Discussion

Carbapenems are the most frequently used antibiotics for the treatment of multidrug-resistant *Enterobacteriaceae* infections. Few therapeutic options are available for treatment of carbapenemase-producing *Enterobacteriaceae*, especially for *Providencia* spp. isolates that are intrinsically resistant to polymyxins.

Although bla_{NDM-1} has been described in Brazil recently (5), we observed that reports by clinical laboratories of isolates possessing this enzyme became more frequent after the implementation of the ANVISA technical standards. This technical document recommends MBL screening of all carbapenem-resistant *Enterobacteriaceae*, using carbapenem plus carbapenem/EDTA.

Previous studies detected the association of NDM-1 with other carbapenemases and methylase genes (13-16). In Brazil, Bueno and colleagues (16) recently described the coproduction of the *rmt*G methylase in *K. pneumoniae* isolates that carried bla_{KPC-2} and $bla_{CTX-M-15}$. To the best of our knowledge, this study is the first to describe the association of bla_{NDM-1} and bla_{KPC-2} in Brazilian isolates, as well as the association of bla_{NDM-1} with *arm*A, a 16S rRNA methylase gene. Moreover, this is the first report of *arm*A among Brazilian clinical isolates.

The emergence of bla_{NDM-1} in association with other resistance genes demonstrates the rapid evolution of bacteria and the ability to acquire and keep different resistance genes. This fact is worrisome because the antimicrobial options available for treatment of these infections are decreasing. The distinct patterns observed among ECL clinical isolates suggest that bla_{NDM-1} had spread silently in the hospitals.

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