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Characterization of Transformants Obtained From NDM-1–Producing *Enterobacteriaceae* in Brazil

To the Editor—The emergence of carbapenemase-producing Enterobacteriaceae (CPE) is an important public health problem because the treatment of CPE is difficult; few options remain available for clinical use.[1] The New Delhi metallo-β-lactamase (NDM-1) is the most common class B carbapenemase among Enterobacteriaceae, and it has been detected increasingly frequently in several countries,[2] including Brazil.[3–8] The aim of this study was to evaluate the characteristics of transformants obtained from NDM-1–production in different bacterial species of *Enterobacteriaceae* identified in southern Brazil.

Isolates were selected from a surveillance study that evaluated *Enterobacteriaceae* with reduced susceptibility to carbapenems in Rio Grande do Sul State, southern Brazil. A total of 9 clinical NDM-producing isolates from 4 hospitals were selected for this study: 3 *Klebsiella oxytoca*, 2 *Enterobacter clocae* complex, 1 *Klebsiella pneumoniae*, 1 *Morganella morganii*, 1 *Escherichia coli*, and 1 *Citrobacter freundii*. These isolates were initially identified by the VITEK2 system (bioMèrieux, Marcy-l'Étoile, France) and confirmed by 16S rRNA sequencing. The bla_{NDM} gene was detected by a multiplex real-time polymerase chain reaction (PCR), which also included primers for the bla_{KPC}, bla_{VIM}, bla_{GES}, bla_{NDM}, bla_{OXA-48}, and bla_{IMP} genes.[9] The presence of bla_{NDM} was further confirmed by conventional PCR, and the amplicons were purified and sequenced using a BigDye Terminator Kit (version 3.1, Thermo Fisher Scientific, Waltham, MA) and an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). GenBank was used to access the bla_{NDM} sequences deposited to date, and the BioEdit program was used to compare similarities between sequences. The plasmids were extracted by alkaline lysis and were transformed into *E. coli* TOP10 electrocompetent cells by electroporation. Transformants were selected on Luria-Bertani agar containing 2 μg/mL ceftazidime. The transformants were evaluated for the bla_{NDM} gene by conventional PCR with specific primers. The minimum inhibitory concentrations (MICs) of imipenem, meropenem, doripenem, piperacillin/tazobactam, ceftriaxone, cefepime, aztreonam, gentamicin, amikacin, polymyxin, and tigecycline were assessed by Etest (bioMèrieux, Marcy-l'Étoile, France). The modified Hodge test (MHT) and the combination-disc test (ie, meropenem and imipenem with and without ethylenediaminetetraacetic acid [EDTA]) were used as phenotypic methods for carbapenemase and metallo-β-lactamase detection, respectively.

It was possible to obtain transformants from all 9 clinical isolates. The transformants obtained from each isolate presented higher MICs than the original *E. coli* TOP10 for β-lactams. In fact, the MICs of transformants were similar to those of the donor NDM-positive clinical isolates, which showed high levels of resistance (Table 1).

Notably, the combined-disc assay with EDTA proved to be positive (ie, EDTA inhibited the carbapenem activity) for all NDM-1–producing clinical isolates and transformants.

Plasmid analysis indicated that most transformants contained a 110-kb plasmid: 2 from the *E. clocae* complex and 1 each from *K. oxytoca*, *M. morganii*, *E. coli*, and *C. freundii*. However, it was also possible to identify the presence of a 52-kb plasmid in a transformant from *K. oxytoca*, a 154-kb plasmid from a *K. oxytoca*, and a *K. pneumoniae* (Table 1).

TABLE 1. Phenotypic Characterization of Isolates and Transformants Obtained From NDM-1–Producers in Brazil

				Phenotypic Characterization														
				Minimum Inhibitory Concentration (μg/mL)										EDTA				
Isolate	Bacteria	Hospital	Specimen	IMP	MEM	ERT	PTZ	CRO	CPM	AZT	GEN	AMK	POL	TGC	MHT	IPM	MEM	Estimated Plasmid Size, Kb
821F	*Enterobacter cloacae*	1	Cerebrospinal fluid	12	>32	>32	>256	>32	192	32	>256	>256	0.38	0.38	Pos	Pos	Pos	110
T 821F				>32	16	6	>256	>32	24	0.19	0.75	4	0.5	0.38	Neg	Pos	Pos	110
871F	*Morganella morganii*	2	Rectal swab	>32	24	6	>256	>32	16	3	128	12	>1024	4	Neg	Pos	Pos	66–110
T 871F				>32	>32	6	>256	>32	96	0.094	0.75	2	0.38	0.25	Neg	Pos	Pos	110
2007F	*E. cloacae*	3	Rectal swab	6	>32	12	>256	>32	>256	96	96	8	0.5	1.5	Pos	Pos	Pos	110
T 2007F				>32	>32	32	>256	>32	>256	3	>256	2	0.75	0.19	Pos	Pos	Pos	110
2610F	*Escherichia coli*	3	Rectal swab	16	>32	16	>256	>32	192	>256	0.75	3	0.38	1.5	Neg	Pos	Pos	66–110
T 2610F				>32	>32	>32	>256	>32	24	>256	0.25	1	0.5	0.25	Pos	Pos	Pos	110
2612F	*Citrobacter freundii*	3	Urine	12	12	32	>256	>32	>256	96	16	3	0.5	1.5	Neg	Pos	Pos	66–110
T 2612F				6	6	3	>256	>32	24	0.125	0.38	2	0.25	0.25	Neg	Pos	Pos	110
2748F	*Klebsiella oxytoca*	4	Rectal swab	12	32	16	>256	>32	48	12	12	32	0.5	0.38	Neg	Pos	Pos	52–110
T 2748F				4	3	2	128	>32	8	0.19	0.5	2	0.5	0.25	Neg	Pos	Pos	52
2763F	*K. oxytoca*	3	Rectal swab	>32	>32	>32	>256	>32	>256	>256	6	1.5	0.38	6	Pos	Pos	Pos	66–110–154
T 2763F				>32	16	>32	>256	>32	24	96	0.5	2	0.38	0.25	Pos	Pos	Pos	154
3035F	*K. pneumoniae*	3	Urine	4	4	4	>256	>32	16	3	0.5	1.5	1.5	0.75	Neg	Pos	Pos	66–110–154
T 3035F				4	2	3	>256	>32	8	0.19	1	2	0.38	0.25	Neg	Pos	Pos	154
3116F	*K. oxytoca*	3	Urine	8	>32	>32	>256	>32	48	>256	0.25	1.5	0.19	1.5	Neg	Pos	Pos	66–110
T 3116F				4	3	3	192	>32	12	0.094	0.25	2	0.25	0.25	Neg	Pos	Pos	110
	E. coli TOP10	NA	NA	0.25	0.032	0.008	3	0.25	0.047	0.0125	0.5	2	≤0.125	0.06	NA	NA	NA	NA

NOTE. T, transformant; MHT, modified Hodge test; EDTA, ethylenediaminetetraacetic acid; Neg, negative; Pos, positive; NA, not applicable; IMP, imipenem; MEM, meropenem; ERT, ertapenem; PTZ, piperacillin/tazobactam; CRO, ceftriaxone; CPM, cefepime; AZT, aztreonam; GEN, gentamicin; AMK, amikacin; POL, polymyxin B; TGC, tigecycline.

The in vitro transfer of plasmids containing the bla_{NDM-1} gene in our study confirms that this carbapenemase gene can be readily mobilized among different species of *Enterobacteriaceae*. Moreover, *E. coli* TOP10 transformants containing the bla_{NDM-1} gene presented similar characteristics of the original clinical isolate, with increased MIC to β-lactams and positive results of the combined-disc assay with EDTA. Although a plasmid of the same molecular weight (~110 bp) was observed in 6 of 9 transformants, the identification of other plasmids (~52 bp and ~154 bp) suggests that the bla_{NDM-1} gene is located in different mobile genetic elements.

Molecular investigations involving both the characterization of isolates of NDM-positive bacteria and the characterization of the plasmids containing bla_{NDM-1} genes reveal a highly complex picture. The plasmids encoding NDM also appear highly heterogeneous based on molecular size, incompatibility type, and linked antibiotic-resistance genes.[2] Moreover, our data support the findings from Brazil in which a variety of plasmids were found. The gene bla_{NDM-1} was identified on plasmid with an estimated size of 420–490 kb in *Enterobacter hormaechei*.[8] In *Enterobacter cloacae*, *Providencia rettgeri*, and *Klebsiella pneumoniae*, the plasmid was reported to be ~230 kb.[9] *Escherichia coli* and *Enterobacter hormaechei* had plasmid sizes of 70 kb and 90 kb, respectively.[10] The plasmid size in *Acinetobacter baumannii* was 100 kb.[7]

In summary, the results of this study demonstrate the variety of plasmids observed in the transformants and suggests that strains producing bla_{NDM-1} harbor plasmids of different sizes, demonstrating the plasticity of these mobile genetic elements. These findings highlight the need for continuous monitoring of the presence of carbapenemases. Our results contribute to the understanding of carbapenem resistance in *Enterobacteriaceae* and to the molecular characterization of NDM-1–producing isolates in Brazil.

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Is AGREE II a counsel of perfection? A letter commenting on Lytvyn et al[1]

To the Editor—We read the systematic survey (review) of *Clostridium difficile* (CD) guidelines (August 2016) with interest. We suggest that Lytvyn et al are proposing a counsel of perfection, ignoring the realities of producing practical guidelines to address rising infection levels. In particular, we question their data extraction from the UK guidelines and