Journal of Antimicrobial Chemotherapy Advance Access published April 25, 2013

Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother
doi:10.1093/jac/dkt124

Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico

Humberto Barrios[1]†, Ulises Garza-Ramos[1]†, Fernando Reyna-Flores[1], Alejandro Sanchez-Perez[1], Teresa Rojas-Moreno[1], Elvira Garza-Gonzalez[2], Jorge Martín Llaca-Diaz[3], Adrian Camacho-Ortiz[4], Santos Guzmán-López[5] and Jesus Silva-Sanchez[1]*

[1]Instituto Nacional de Salud Pública (INSP), CISEI, Cuernavaca, Morelos, México; [2]Servicio de Gastroenterología, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Nuevo León, México; [3]Departamento de Patología Clínica, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Nuevo León, México; [4]Servicio de Infectología, Hospital Universitario Dr. José Eleuterio González, Universidad Autónoma de Nuevo León, Nuevo León, México; [5]Departamento de Anatomía, Facultad de Medicina, Universidad Autónoma de Nuevo León, Nuevo León, México

*Corresponding author. Tel: +52-7773-29-30-21; Fax: +52-7773-17-54-85; E-mail: jsilva@insp.mx
†H. Barrios and U. Garza-Ramos have equally contributed to the manuscript.

Keywords: antimicrobial resistance, Enterobacteriaceae, Gram-negative

Sir,

Bacteria of the genus *Providencia* are Gram-negative opportunistic pathogens that have been isolated from a wide variety of environments, including human stool samples. They comprise part of the natural human gut flora but may also cause infections, including travellers' diarrhoea.[1] They are also responsible for urinary tract and other nosocomial infections in humans.[2] The New Delhi metallo-β-lactamase (NDM-1) is the most recently discovered transferable molecular class B metallo-β-lactamase. The gene encoding this enzyme was located on a 178 kb plasmid belonging to incompatibility group A/C in a *Providencia stuartii* clinical isolate.[3] However, it has been described in different plasmid types (IncA/C, IncF, IncL/M, IncN or untypeable) and is also chromosomally integrated.[4]

This work describes four *Providencia rettgeri* clinical isolates obtained from patients with urinary tract infection in the intensive care unit (ICU) of the University Hospital of Monterrey, Mexico, between January and June 2012 (Table 1). The *P. rettgeri* isolates were identified using the API 20E galleries (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (TEK Diagnostic Systems Inc., Cleveland, OH, USA). In addition, the 16S rRNA gene was characterized by using primers previously described.[5] A possible epidemiological link between the patients was a surgical resident involved in the care of the four patients while in the ICU. None of the patients had evidence of infection prior to being transferred to the ICU; the average time to positive urine cultures for *P. rettgeri* was 29 days (range 12–68 days).

MICs were determined by broth microdilution following CLSI recommendations[6] and the phenotypic screening to determine the production of a carbapenemase enzyme was carried out using a double-disc synergy test (meropenem, imipenem and EDTA).[6] The isolates were screened using PCR for genes encoding a range of carbapenemase enzymes, including KPC, GES, IMP, SIM, GIM, SPM, VIM and NDM.[7] Genotyping was performed using PFGE and the results were analysed following the guidelines of Tenover *et al.*[8] using GelCompar II (Applied Math, Kortrijk, Belgium). The plasmid profile was analysed using ion-interchange columns (Qiagen, Valencia, CA, USA); subsequently, a Southern hybridization was carried out with a non-radioactive probe for the NDM-1 gene. Mating and transformation experiments and identification of plasmid incompatibility groups by PCR replicon typing were undertaken as described previously.[9,10]

The genotyping analysis of the four *P. rettgeri* isolates showed one clonal group (data not shown). All isolates were susceptible to tigecycline (1 mg/L), but resistant to imipenem, meropenem, piperacillin, ceftazidime, cefotaxime, ciprofloxacin and colistin (Table 1). The isolates were positive for carbapenemase activity and the PCR assays and sequencing demonstrated the presence of the gene encoding NDM-1. According to the Southern hybridization (data not shown) and PCR replicon typing results obtained with the transconjugants and recombinants, the NDM-1 gene was identified on a 310 kb IncK plasmid (Table 1). However, the mating and the transformation experiments showed the respective transconjugant (310 kb and 160 kb) and transformant (310 kb and 50 kb) with two different plasmids harboured in the clinical isolates (Table 1). Similar results have been recently described suggesting that a helper plasmid is necessary for the mobilization of the plasmid-borne NDM-1.[11] In this work, the 160 kb plasmid could be playing the role of helper. This is the first known report of an NDM-1-producing *P. rettgeri* in Mexico. This finding points to the need to enforce the molecular epidemiological surveillance of these pathogens and enzymes in order to prevent their dissemination among hospitals as well as to other bacterial genera.

Funding

This work was supported by grants CB-2009-01-136339 from CONACyT (Mexican Council for Science and Technology). Rio Arronte provided the sequencing equipment.

Transparency declarations

None to declare.

Table 1. Characteristics of metallo-β-lactamase NDM-1-producing *P. rettgeri* isolates

Isolate	Patient	Patient gender/age (years)	Date of admission/time in hospital (days)	Number of days after admission that *P. rettgeri* was isolated	Plasmids (kb)[a]	Incompatibility group, Inc	MIC (mg/L)							
							IPM	MEM	CIP	GEN	PIP	CAZ	CTX	TGC	CST
06-1617	2[b]	male/22	January 2012 (27)	17	**310**, **160**, <40	K	>256	>256	64	>256	>256	>256	>256	1	>32
06-1619	3[c]	male/16	January 2012 (21)	12	**310**, **160**, <40	K	>256	>256	32	>256	>256	>256	>256	1	>32
06-1622	1[d]	female/50	January 2012 (70)	68	310, 130, 50	K	>256	>256	64	4	>256	>256	>256	1	>32
06-1623	4[e]	female/53	May 2012 (31)	21	310, 130, 50	K	>256	>256	64	4	>256	>256	>256	1	>32

IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; PIP, piperacillin; CAZ, ceftazidime; CTX, cefotaxime; TGC, tigecycline; CST, colistin.
[a]The plasmids appearing in bold or underlined correspond to the plasmids obtained in the mating and transformation experiments, respectively.
[b]The patient died of non-infectious complications.
[c]The patient died of neurological complications.
[d]The patient was transferred to a community hospital 10 days after isolation with no signs of infection.
[e]The patient was discharged without clinical signs of infection and remained asymptomatic 6 weeks after discharge.

References

1 Yoh M, Matsuyama J, Ohnishi M *et al*. Importance of *Providencia* species as a major cause of travellers' diarrhoea. *J Med Microbiol* 2005; **54**: 1077–82.

2 O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev* 2000; **13**: 534–46.

3 McGann P, Hang J, Clifford RJ *et al*. Complete sequence of a novel 178-kilobase plasmid carrying bla_{NDM-1} in a *Providencia stuartii* strain isolated in Afghanistan. *Antimicrob Agents Chemother* 2012; **56**: 1673–9.

4 Nordmann P, Poirel L, Walsh TR *et al*. The emerging NDM carbapenemases. *Trends Microbiol* 2011; **19**: 588–95.

5 Pei Z, Bini EJ, Yang L *et al*. Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci USA* 2004; **101**: 4250–5.

6 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Ninth Edition: Approved Standard M07-A9*. CLSI, Wayne, PA, USA, 2012.

7 Mulvey MR, Grant JM, Plewes K *et al*. New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada. *Emerg Infect Dis* 2011; **17**: 103–6.

8 Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

9 Garza-Ramos U, Barrios H, Hernandez-Vargas MJ *et al*. Transfer of quinolone resistance gene *qnrA1* to *Escherichia coli* through a 50 kb conjugative plasmid resulting from the splitting of a 300 kb plasmid. *J Antimicrob Chemother* 2012; **67**: 1627–34.

10 Carattoli A, Bertini A, Villa L *et al*. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods* 2005; **63**: 219–28.

11 Poirel L, Dortet L, Bernabeu S *et al*. Genetic features of bla_{NDM-1}-positive Enterobacteriaceae. *Antimicrob Agents Chemother* 2011; **55**: 5403–7.