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Letter to the Editor

Emergence of genetically related NDM-1-producing *Providencia rettgeri* strains in Argentina

Sir,

New Delhi metallo- β -lactamase 1 (NDM-1) was initially identified in *Escherichia coli* and *Klebsiella pneumoniae* isolates in Sweden from a patient previously hospitalised in India [1]. According to the Antimicrobial Resistance Surveillance Network (Pan American Health Organization/World Health Organization), NDM-producers in Latin America have been scarce and associated with species of Enterobacteriaceae from Guatemala, Mexico, Colombia, Brazil, Uruguay and Costa Rica, although in Honduras and Paraguay it was reported in *Acinetobacter*. (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=24472; accessed 17.06.14). Here we report the emergence of NDM-1 carbapenemase in Argentina.

Since 2005, we designed an algorithm to detect carbapenemases in species of Enterobacteriaceae at the level of the clinical microbiology laboratory, which was implemented by 432 laboratories across Argentina (National Quality Control Program in Bacteriology, Argentinean Ministry of Health) (<http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/Ppt0000011.pdf>; accessed 17.06.14). Briefly, metallo- β -lactamase (MBL) production is suspected in isolates that exhibit decreased susceptibility to carbapenems [Clinical and Laboratory Standards Institute (CLSI) criteria] and a positive synergism between ethylene diamine tetraacetic acid (EDTA) and carbapenem disks [2].

During 2013, following this algorithm, a hospital from Buenos Aires City detected a MBL phenotype in two *Providencia rettgeri* isolates [strains were first identified using the VITEK[®] 2 system (bioMérieux, Lyon, France)]. The first case was a 54-year-old male patient with underlying vascular disease admitted due to the poor outcome of a surgical wound. After 3 month of hospitalisation, *P. rettgeri* M15628 was recovered from a catheter and was assumed to be a colonising isolate (concomitant blood cultures were negative). A rectal swab plated on chromogenic medium (CHROMagar[™] KPC; CHROMagar, Paris, France) revealed gastrointestinal colonisation by KPC-producing *K. pneumoniae* but not NDM-producers. The patient was discharged alive after an additional month of hospitalisation. Four days later, a 56-year-old male patient was admitted to a different unit of the same medical ward because of terminal prostate cancer. Forty-eight days after admission, *P. rettgeri* M15758 was recovered from a urine culture obtained by an indwelling urinary catheter. Carbapenem-resistant pathogens were not detected from a rectal swab plated on CHROMagar KPC. The patient was successfully treated with amikacin, however he died 13 days later due to disseminated cancer.

Strains were submitted to the National Reference Laboratory for further phenotypic and molecular characterisation. Strains were

confirmed as *P. rettgeri* by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker, Billerica, MA). Antimicrobial susceptibility (Table 1) revealed that the strains were resistant to almost all tested agents but remained susceptible to aminoglycosides (excluding the production of 16S rRNA methylases). EDTA reduced the carbapenem minimum inhibitory concentrations (MICs) by at least three dilutions in both strains, suggesting the presence of MBLs. M15628, but not M15758, was resistant to aztreonam, suggesting the co-production of an extended-spectrum β -lactamase (ESBL). The modified Hodge test was (weakly) positive with carbapenem for both isolates.

In both isolates, PCR screening followed by DNA sequencing detected the presence of *bla*_{NDM-1}. In addition, *bla*_{PER-2} was detected in M15628. PCRs targeting *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{OXA-48} and *bla*_{CTX-M} were negative. PCR mapping and sequencing of a 1.7-kb fragment surrounding *bla*_{NDM-1} from both strains revealed 100% identity with the sequence reported for *Acinetobacter pittii* (accession no. KF285829.1) where *bla*_{NDM-1} is inserted in the composite transposon Tn125. *Sma*I pulsed-field gel electrophoresis (PFGE) studies revealed that the *P. rettgeri* isolates were highly related (one band difference in the macrorestriction pattern).

Transfer of *bla*_{NDM-1} by biparental conjugation to sodium azide-resistant *E. coli* J53 was unsuccessful. S1 nuclease digestion showed that M15628 harboured two plasmids (142 kb and 325 kb) and M15758 harboured four plasmids (62, 79, 217 and 312 kb). The *bla*_{NDM-1} probe hybridised with the 325, 217 and 312 kb plasmids, respectively. By PCR, plasmid replicon typing of M15628 was positive for B/O, and M15758 was non-typeable [3]. Unlike Brazilian *P. rettgeri* isolates where *bla*_{NDM-1} was chromosomally integrated [4], the observation in this work of different plasmids harbouring *bla*_{NDM-1} suggests a complex dynamic in the dissemination of *bla*_{NDM-1} in Argentina.

P. rettgeri has recently been recognised as a key organism for the dissemination of NDM in the Southern Cone as it has been involved in the emergence of this carbapenemase in Uruguay and Brazil [4]. The isolates characterised in this work are the first NDM-1-producers detected in Argentina. The origin of NDM-1 in Argentina remains unclear, since no history of travel was established for either patient. The finding of *bla*_{NDM-1} in *P. rettgeri* strains with a homogeneous clonal background and its isolation from patients admitted 81 days apart from each other suggests clone persistence within the hospital. Despite this ability, no further cases were observed in this hospital. However, 3 months after the second case, a *bla*_{NDM-1} + *bla*_{PER-2}-producing *P. rettgeri* belonging to the same clonal type (not shown) was recovered from a new patient admitted to a different hospital in Buenos Aires, with no history of admission to the index institution.

In conclusion, we confirmed the active circulation of NDM carbapenemase in Argentina, mobilised by the escalation of a single *P. rettgeri* clone. Thus, there is an urgent need to adopt

Table 1
Antimicrobial susceptibility of NDM-producing *Providencia rettgeri* clinical isolates.

	MICs (mg/L) ^a	
	M15628 (<i>bla</i> _{NDM-1} + <i>bla</i> _{PER-2})	M15758 (<i>bla</i> _{NDM-1})
Imipenem ^b	8	8
Imipenem/EDTA ^{b,c}	1	0.5
Meropenem ^b	8	8
Meropenem/EDTA ^{b,c}	0.12	0.12
Ertapenem ^b	4	4
Aztreonam ^b	16	≤0.03
Cefoxitin ^b	32	32
Cefotaxime ^b	256	64
Ceftazidime ^b	>256	>256
Cefepime ^b	64	16
Ampicillin/sulbactam	>16	>16
Piperacillin/tazobactam	>64	64
Gentamicin	≤1	≤1
Amikacin	≤2	≤2
Nalidixic acid	>16	>16
Ciprofloxacin	>2	>2
Trimethoprim/sulfamethoxazole	>16	>16
Nitrofurantoin	256	256
Colistin	>16	>16

MIC, minimum inhibitory concentration; EDTA, ethylene diamine tetra-acetic acid.

^a Antimicrobial susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) standards (M100-S24).

^b MICs were determined using agar dilution; MICs of other antibiotics were determined using the VITEK[®] 2C (AST-N082 card) (bioMérieux, Lyon, France).

^c EDTA at a fixed concentration of 0.4 mM. The *bla*_{VIM-11}-producer *Pseudomonas aeruginosa* M5109 was used for quality control purposes [5].

measures for timely control of the spread of this clone. Comparison of *P. rettgeri* isolates from Uruguay, Brazil and Argentina becomes necessary to understand the dynamics of dispersion of NDM-1-producing *P. rettgeri* strains in the Southern Cone.

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Competing interests

None declared.

Ethical approval

Not required.

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