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Brief report

Emergence of *Providencia rettgeri* NDM-1 in two departments of Colombia, 2012–2013[☆]



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ABSTRACT

Introduction: In Colombia, between 2012 and 2013, 19 isolates with NDM were identified, of which 14 corresponded to *Providencia rettgeri*.

Methods: The isolates were identified by Vitek-2, and antimicrobial susceptibility was evaluated by broth microdilution. The carbapenemase phenotypes were determined with Modified Hodge Test and synergy tests with EDTA/SMA and APB, the genotypes by PCR using specific primers for KPC, GES, IMP, VIM, OXA-48 and NDM, and genetic relationships were established with DiversiLab.

Results: The isolates were resistant to carbapenems, third-generation cephalosporins, piperacillin-tazobactam, amikacin, gentamicin and tigecycline, except aztreonam. All isolates were positive for EDTA/SMA and NDM-1, and negative for APB and other carbapenemases. Two genetic groups, group 1 (n=9 isolates), group 2 (n=4 isolates) and an isolate defined as not genetically related.

Conclusion: This work describes the circulating of NDM-1-producing *P. rettgeri* in Colombia.

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Emergencia de *Providencia rettgeri* NDM-1 en dos departamentos de Colombia, 2012–2013

RESUMEN

Introducción: En Colombia, entre 2012 y 2013 se identificaron 19 aislamientos con NDM, de los cuales 14 correspondían a *Providencia rettgeri*.

Métodos: Los aislamientos se identificaron con Vitek-2, y la sensibilidad antimicrobiana se evaluó por microdilución en caldo. Las carbapenemasas se determinaron fenotípicamente con test de Hodge modificado y pruebas de sinergia con EDTA/SMA y APB, genotípicamente por PCR usando iniciadores específicos para KPC, GES, IMP, VIM, OXA-48 y NDM, y las relaciones genéticas se establecieron con DiversiLab.

Resultados: Los aislamientos fueron resistentes a carbapenémicos, cefalosporinas de tercera generación, piperacilina-tazobactam, amikacina, gentamicina y tigeciclina, excepto a aztreonam. Todos los aislamientos fueron positivos para EDTA/SMA y NDM-1 y negativos para APB y otras carbapenemasas. Se definieron 2 grupos genéticos: grupo 1 (n=9 aislamientos), grupo 2 (n=4 aislamientos), y un aislamiento no relacionado genéticamente.

Conclusión: Este trabajo describe la circulación de grupos de *P. rettgeri* productores de NDM-1 en Colombia.

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Palabras clave:

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Introduction

Providencia rettgeri is one of the most clinically significant species of the genus *Providencia* spp. This opportunistic pathogen causes multiple infections, such as: urinary tract infections, gastrointestinal infections and bacteraemia, among others.¹ NDM was first described in January 2008, and this enzyme has spread rapidly worldwide, being detected mainly in enterobacteria and, to a lesser extent, in *Acinetobacter* spp. and *Pseudomonas* spp.²

In Colombia, NDM was first described in 2012, in isolates of *Klebsiella pneumoniae* that triggered an outbreak.³ From this report until December 2013, the National Institute of Health (INS) surveillance program for antimicrobial resistance in microorganisms causing healthcare-associated infections (HCAI), through the Microbiology Group (National Reference Laboratory), has confirmed 19 new cases of NDM (14 *P. rettgeri*, 3 *Acinetobacter baumannii*, one *Pseudomonas aeruginosa* and one *K. pneumoniae*, the latter a co-producer of NDM and KPC), observing that *P. rettgeri* NDM equals more than 70% of NDM-producing isolates in Colombia.

In consideration of the above, the objective of this report is to describe the microbiological and molecular characteristics of *P. rettgeri* NDM identified between 2012 and 2013 in Colombia.

Materials and methods

History

The INS surveillance program for antimicrobial resistance in microorganisms that cause HCAI monitors various events of interest in public health, and includes the national laboratory surveillance of carbapenem resistance, which began in September 2012. From that date until December 2013, 749 isolates have been received (483 enterobacteria, 211 *Pseudomonas* spp. and 55 *Acinetobacter* spp.) for confirmation of carbapenemases, and these enzymes were detected in 529 (70.6%) isolates (343 enterobacteria, 136 *Pseudomonas* spp. and 50 *Acinetobacter* spp.). The most detected carbapenemase in enterobacteria was KPC (90.1%; n = 309 isolates), in *Pseudomonas* spp. was VIM, followed by KPC (VIM: 70.6%; n = 96 isolates, and KPC: 22.1%; n = 30 isolates), and in *Acinetobacter* spp. was OXA-23 (86%; n = 43 isolates); the cases of NDM in this period were 3.6% (n = 19), and of these 73.7% (n = 14) were *P. rettgeri*.

Bacterial isolates

The INS Microbiology Group received 14 isolates of *P. rettgeri*, recovered in 5 hospitals of 3 cities located in 2 departments: hospital H1 (Bucaramanga-Santander), hospital H2 “outpatient centre” (Madrid-Cundinamarca) and hospitals H3–H5 (Bogotá D.C.-Cundinamarca).

Phenotypic characterisation

Identification at the genus and species level was confirmed using Vitek-2 (bioMérieux, Marcy l'Etoile, France) and antimicrobial susceptibility was assessed using broth microdilution (Sensititre panels; TREK Diagnostic Systems, Westlake, Ohio, USA). The results obtained were interpreted according to the parameters established by the CLSI.⁴ Except for tigecycline, for which the EUCAST criteria were used (breakpoints available at http://www.eucast.org/clinical_breakpoints/version_5.0). The phenotypic detection of carbapenemases was performed using: (a) modified Hodge test (MHT) (4), and (b) double-disc synergy tests using specific inhibitors for class B, carbapenemases (ethylenediaminetetraacetic acid/mercaptoacetic acid [EDTA/SMA]), and for class A, carbapenemases (Phenylboronic acid [PBA]).

Genotypic characterisation

The detection of beta-lactamase encoding genes was performed by conventional PCR, for *bla*_{KPC}, *bla*_{GES}, *bla*_{VIM},⁵ *bla*_{NDM},⁶ *bla*_{IMP},⁷ *bla*_{OXA-48},⁸ *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV}⁹; both chains of the carbapenemase amplification products were sequenced using an automated sequencer (MacroGen sequencing service, Korea, ABI PRISM® 3730XL from Applied Biosystem). Genotyping was performed using Diversilab (bioMérieux, Marcy l'Etoile, France) using the bacterial kit, and the results were analysed with the Kullback Leibler correlation (based on the presence and absence of bands rather than intensity); isolates with similarity >95% were considered indistinguishable; with similarity of 93–95%, closely related, and with similarity <93%, different.

Results

Bacterial isolates

P. rettgeri was recovered in patients ranging in age from 16 to 88 years, being primarily recovered in men (85.7%; n = 12) and in urine samples (71.4%; n = 10) associated with both infection and colonisation (Table 1). Nine isolates came from the H1 hospital, most of them classified as colonisation (n = 6), isolated in different departments of the institution, between 2012 and 2013 (Table 1). The remaining 5 isolates were isolated in hospitals H2 to H5 in 2013, with one isolation per institution, except H3, which had 2 isolates (Table 1).

Phenotypic characterisation

The isolates were resistant to all antimicrobials evaluated; carbapenems, third generation cephalosporins, piperacillin-tazobactam, amikacin, gentamicin and tigecycline, except aztreonam (Table 1). All isolates were positive for EDTA/SMA, negative for PBA, and only 4 isolates were positive for MHT (Table 1).

Genotypic characterisation

All isolates were positive for NDM-1, 13 for TEM and 4 for CTX-M (Table 1). No isolate amplified KPC, GES, IMP, VIM, OXA-48 or SHV. Classification with Diversilab helped define 2 clonal groups of 9 (group 1) and 4 (group 2) isolates, respectively (Fig. 1), and one isolate that is not genetically related to the rest. Group 1 is composed of 9 isolates (8 indistinguishable and one closely related), all from the H1 hospital, while group 2 consists of 3 indistinguishable isolates and one closely related from hospitals H2 to H5 (Fig. 1). All isolates of group 2 were positive for CTX-M.

Discussion

In Colombia, various types of carbapenemases are circulating, and it is considered an endemic region for KPC carbapenemases, detected mainly in enterobacteria and *Pseudomonas* spp.,^{10,11} VIM mainly identified in *Pseudomonas* spp.,¹¹ and OXA-23 in *Acinetobacter baumannii*.¹² Although in Colombia NDM was detected just under 5 years ago,³ currently its identification has increased in the country according to INS surveillance data (these data are not shown), which is a matter of great concern due to the rapidity of dissemination of this carbapenemase worldwide and the possibility that in our country this mechanism of resistance may become of high prevalence, as has happened with other carbapenemases.

In Latin America, NDM was detected for the first time in isolates of *Klebsiella pneumoniae* in Guatemala¹³ and Colombia³; at

Table 1
Clinical, phenotypic and genotypic characteristics of the isolates.

Isolate	Isolation date (month/year)	Hospital	Patient (sex/age in years)	Infection/ colonisation	Service	Sample	Minimum inhibitory concentration (µg/ml)										Phenotypic tests			Beta- lactamases	
							IMP	MEM	ERT	DOR	AZT	CAZ	CTX	PTZ	GEN	AMK	TGC	MHT	EDTA/SMA		PBA
RB-110	Nov 12	H1	M/16	Colonisation	Burn Unit	Urine	≥8	8	2	8	≤2	16	64	≥128/4	≥16	≥64	2	Neg	Pos	Neg	NDM, TEM
RB-111	Nov 12	H1	M/75	Colonisation	Outpatient	Urine	≥8	4	2	4	≤2	16	32	≥128/4	≥16	≥64	2	Neg	Pos	Neg	NDM, TEM
RB-151	Jan 13	H1	F/58	Colonisation	A&E	Urine	≥8	4	4	8	≤2	≥32	64	≥128/4	≥16	≥64	2	Neg	Pos	Neg	NDM, TEM
RB-152	Dec 12	H1	M/72	Colonisation	A&E	Bronchial secretion	≥8	≥8	4	≥8	≤2	32	≥64	≥128/4	≥16	≥64	8	Neg	Pos	Neg	NDM, TEM
RB-153	Jan 13	H1	M/38	Colonisation	A&E	Urine	≥8	4	2	≥8	≤2	16	64	≥128/4	≥16	≥64	4	Neg	Pos	Neg	NDM, TEM
RB-350	ND	H2	M/73	Colonisation	ND	Urine	≥8	≥8	4	≥8	≤2	32	≥64	≥128/4	≥16	≥64	4	Neg	Pos	Neg	NDM, TEM, CTX-M
RB-351	Mar 13	H3	M/77	Infection	Internal Medicine	Urine	4	≤1	1	1	≤2	16	16	≥128/4	8	≤4	2	Neg	Pos	Neg	NDM
RB-352	Mar 13	H4	M/88	Infection	Internal Medicine	Urine	≥8	≥8	≥4	≥8	≤2	≥32	≥64	≥128/4	≥16	≥64	4	Neg	Pos	Neg	NDM, TEM, CTX-M
RB-353	Apr 13	H3	M/60	Infection	Internal Medicine	Urine	≥8	≥8	≥4	≥8	≤2	≥32	≥64	≥128/4	≥16	32	8	Neg	Pos	Neg	NDM, TEM, CTX-M
RB-354	Jan 13	H5	M/56	Infection	Orthopaedics	Surg. Wound Sec.	≥8	≥8	≥4	≥8	≤2	32	≥64	≥128/4	≥16	≥64	8	Neg	Pos	Neg	NDM, TEM, CTX-M
RB-417	May 13	H1	F/51	Infection	Neurosurgery	Urine	≥8	8	4	8	≤2	32	≥64	≥128/4	≥16	≥64	4	Pos	Pos	Neg	NDM, TEM
RB-418	May 13	H1	M/74	ND	A&E	Urine	≥8	≥8	≥4	≥8	≤2	32	≥64	≥128/4	≥16	≥64	2	Pos	Pos	Neg	NDM, TEM
RB-698	Oct 13	H1	M/32	infection	ND	Abdominal secretion	≥8	≤1	0.5	4	≤2	8	16	≥128/4	≥16	≥64	1	Pos	Pos	Neg	NDM, TEM
RB-699	Oct 13	H1	M/18	colonisation	ND	Drainage secretion	≥8	2	0.5	2	≤2	16	32	≥128/4	≥16	≥64	4	Pos	Pos	Neg	NDM, TEM

AMK: amikacin; PBA: synergism test with phenylboronic acid inhibitor; CAZ: ceftazidime; CTX: cefotaxime; DOR: doripenem; EDTA/SMA: synergism test with inhibitor (ethylenediaminetetraacetic acid/mercaptoacetic acid EDTA/SMA; ERT: ertapenem F: female GEN: gentamicin IMP: imipenem M: male Internal med.: internal medicine MEM: meropenem; Neg: negative; Pos: Positive; PTZ: piperacillin-tazobactam; ND: no data; Surg. Wound Sec: Surgical wound secretion; Sec: secretion; TGC: tigecycline; MHT: modified Hodge test; Burn U: Burn unit.

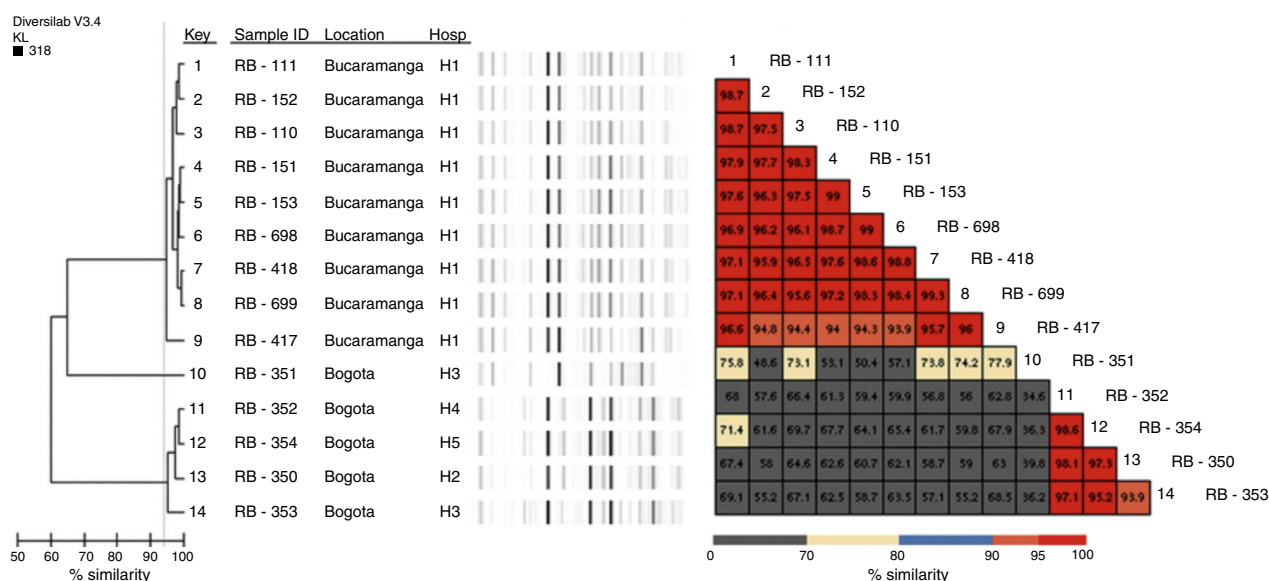


Fig. 1. Dendrogram: image of the virtual gel and similarity matrix of *P. rettgeri* NDM-1 isolates in Colombia classified with Diversilab.

present this enzyme has been isolated in different gram-negative microorganisms in the region, including *P. rettgeri*, which has been identified in Argentina, Brazil, Mexico and Uruguay,¹³ and this is the first report in Colombia. Early detection of carbapenemases in the microbiology laboratory through the use of screening tests is important for preventing the dissemination of these resistance determinants in the hospital setting. In this study it was observed that the identification of class B carbapenemases (NDM) using the test with EDTA/SMA was 100% sensitive and specific, whereas MHT only managed to detect less than 30% of these isolates, which is in agreement with that reported in the literature.^{2,4}

The majority of microorganisms producing carbapenemases have a multiresistance profile, limiting the treatment options, which is why colistin has become one of the antimicrobials of choice to treat these infections.² However, in the case of *P. rettgeri* with NDM, colistin cannot be considered as treatment due to the natural resistance of this microorganism.¹⁴ In this study, the majority of isolates presented a multiresistance phenotype and were only sensitive to aztreonam; this characteristic is being studied as a possible strategy to treat these patients by using a combination of aztreonam and avibactam (betalactamase inhibitor NX104).^{2,15}

This study describes the dissemination of 2 clonal groups; one at the intrahospital level where *P. rettgeri* NDM occurred in an institution in Bucaramanga (H1) and another where *P. rettgeri* NDM and CTX-M were identified in 4 institutions (3 of Bogotá and one of Madrid [city located 28 km from Bogotá]). In Colombia the occurrence of an outbreak by isolates with NDM had been previously described.³ However, this is the first time that the spread of a clonal group has been described in several institutions, and although no relationship was established between patients, it is important to establish permanent and articulated epidemiological and laboratory surveillance in order to take control measures to prevent dissemination of NDM isolates in institutions in these geographical areas.

In conclusion, in Colombia the circulation of 2 genetic groups of *P. rettgeri* NDM-1 is observed. Therefore, it is important to strengthen surveillance at a laboratory level to detect these enzymes, which will allow the necessary measures to be taken to control the spread of these determinants of resistance at the hospital level.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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