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Emergence of genetically unrelated NDM-1-producing *Acinetobacter pittii* strains in Paraguay

Fernando Pasteran¹, Mario Martinez Mora², Ezequiel Albornoz¹, Diego Faccone¹, Rossana Franco², Juana Ortellado³, Nancy Melgarejo², Sonia Gomez¹, Irma Riquelme³, Jorge Matheu⁴, Pilar Ramon-Pardo⁴ and Alejandra Corso¹*

¹Servicio Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas (INEI), ANLIS 'Dr Carlos G. Malbrán', Ciudad Autónoma de Buenos Aires, Argentina; ²Servicio Antimicrobianos, Laboratorio Central de Salud Pública, Asunción, Paraguay; ³Centro Materno Infantil-Hospital de Clínicas, Universidad Nacional de Asunción, San Lorenzo, Departamento Central, Paraguay; ⁴Antimicrobial Resistance and Infection Control Program, International Regulations, Alert and Response and Epidemic Diseases and Water Borne Diseases, Communicable Diseases and Health Analysis (CHA), Pan American Health Organization/World Health Organization, Washington, DC, USA

*Corresponding author. Tel/Fax: +54-11-4303-2812; E-mail: acorso@anlis.gov.ar

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Sir,

The New Delhi metallo-β-lactamase (NDM-1) was initially identified in *Escherichia coli* and *Klebsiella pneumoniae* isolates in Sweden, from a patient previously hospitalized in India. To date, NDM producers in Latin America have been scarce, and associated with species of Enterobacteriaceae from Guatemala, Mexico, Colombia and Brazil, although in Honduras it was reported in *Acinetobacter baumannii*. Here, we report two genetically unrelated NDM-1-producing *Acinetobacter pittii* isolates identified in Paraquay.

Since 1996, the Pan American Health Organization (PAHO) has supported a regional surveillance system, the Antimicrobial Resistance Surveillance Network in Latin America (ReLAVRA), that includes 794 laboratories from 20 Latin American countries, including their respective reference laboratories. This network provides reliable, timely and reproducible microbiological data in order to improve patient care. A regional protocol for the detection of carbapenemases has been harmonized and implemented through ReLAVRA. Briefly, metallo- β -lactamase (MBL) production is suspected in isolates that exhibit decreased susceptibility to carbapenems (CLSI criteria) and a positive synergy test result between a disc containing 10 μ g of imipenem and a disc containing 750 μ g of EDTA plus 1900 μ g of sodium thioglycolate.

During 2012, following the ReLAVRA algorithm, the National Health Laboratory of Paraguay confirmed an MBL phenotype in two Acinetobacter spp. isolates recovered from a single hospital. This phenotype had not previously been observed in Acinetobacter spp. from Paraguay. The first case was a 7-year-old patient admitted in July because of acute encephalitis. After 2 months of hospitalization, an Enterobacter cloacae extendedspectrum β-lactamase producer and Acinetobacter M15274 were recovered from the CSF obtained through a ventricular shunt. The patient received multiple treatment regimens, including trimethoprim/sulfamethoxazole, ciprofloxacin and amikacin, which resulted in clinical and microbiological cure. The patient died after 4 months of hospitalization due to non-infectious causes. The second case was a 2-year-old patient with a diagnosis of acute lymphocytic leukaemia who was admitted in November. Two weeks later the patient developed sepsis, and Acinetobacter M15373 was isolated from a blood culture. The patient showed clinical improvement after treatment with meropenem plus amikacin and was discharged alive after 50 days. Both patients were hospitalized in the same oncology ward but 4 months apart from each other. Remarkably, the patients had no history of travelling.

Strains were submitted to the Regional Reference Laboratory (Servicio Antimicrobianos, INEI, ANLIS 'Dr Carlos G. Malbrán') for further characterization. Strains were identified as *A. pittii* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker, Germany); the 10 most probable database matches were all consistent with *A. pittii*. Antimicrobial susceptibility testing revealed an identical resistance profile in both *A. pittii* isolates, except for ampicillin/sulbactam and quinolones (Table 1). EDTA reduced the carbapenem MICs by at least three dilutions in both strains, suggesting the presence of MBLs (Table 1). The modified Hodge test gave negative results with meropenem but was positive (weakly) with imipenem.

In both isolates, PCR screening followed by DNA sequencing detected the presence of $bla_{\rm NDM-1}$. PCRs targeting other β -lactamase genes ($bla_{\rm VIM}$, $bla_{\rm IMP}$, $bla_{\rm SPM}$, $bla_{\rm KPC}$, $bla_{\rm OXA-23}$, $bla_{\rm OXA-24/40}$, $bla_{\rm OXA-48}$, $bla_{\rm OXA-51}$, $bla_{\rm OXA-58}$, $bla_{\rm OXA-143}$, $bla_{\rm CTX-M}$, $bla_{\rm SHV}$ and $bla_{\rm TEM}$) were negative. Sequencing of a 2.5 kb fragment surrounding $bla_{\rm NDM-1}$ from both strains revealed 100% identity to the sequence reported for $Acinetobacter\ lwoffii$, where ISAba125 was located upstream of $bla_{\rm NDM-1}$, followed by $\Delta trpF$ and $tat.^{9,10}$ The genes that follow to the 3' end, $\Delta groES$, groEL, ISCR27 and the second ISAba25 were PCR mapped, revealing a Tn125 composite transposon as previously reported in $A.\ pittii.^{11}$

ApaI PFGE studies revealed that the *A. pittii* isolates were not clonally related (>10 bands of difference in the macrorestriction pattern). Multilocus sequence typing (MLST) was performed according to the MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/). M15274 and M15373 displayed unique and novel sequence types (STs), designated ST320 and ST321, respectively. M15274 and M15373 shared only the *rplB* allele, confirming that they were genetically unrelated. Furthermore, these STs branched with other reported isolates belonging to the *A. pittii* genomic species, confirming the MALDI-TOF results.

Table 1. Antimicrobial susceptibility (MICs in mg/L)^a of NDM-producing A. pittii clinical isolates and A. baumannii and E. coli transconjugant and recipient strains

	Clinical isolates		Transconjugant strains ^b		Recipient strains	
	A. pittii M15274	A. pittii M15373	A. baumannii M17176	E. coli M15694	A. baumannii ATCC 19606	E. coli J53
Imipenem ^c	>256	>256	>256	1	0.25	0.06
Imipenem/EDTA ^d	0.12	0.12	0.25	0.12	0.25	0.06
Meropenem ^c	>256	>256	>256	0.5	1	0.015
Meropenem/EDTA ^d	0.12	0.25	0.5	0.015	1	0.015
Ertapenem ^c	>256	>256	>256	2	1	0.25
Ampicillin/sulbactam	>16	4	>16	>16	≤1	≤1
Piperacillin/tazobactam	>64	>64	>64	>64	8	≤4
Aztreonam ^c	32	32	32	≤0.03	32	≤0.03
Cefotaxime ^c	>256	>256	>256	32	16	≤0.015
Ceftazidime ^c	>256	>256	>256	>256	8	≤0.06
Cefepime ^c	>256	>256	>256	16	16	0.015
Cefoxitin	>32	>32	>32	>32	≤8	≤8
Gentamicin	≤1	≤1	8	≤1	8	≤1
Amikacin	≤2	≤2	≤2	≤2	≤2	≤2
Nalidixic acid	16	≤2	4	≤2	4	≤2
Ciprofloxacin	1	≤0.25	1	≤0.25	1	≤0.25
Trimethoprim/sulfamethoxazole	≤2	≤2	>256	≤2	>256	≤2
Nitrofurantoin	>256	>256	>256	≤16	>256	≤16
Colistin	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Tigecycline ^c	0.06	0.06	0.25	0.25	0.015	0.015

^aAntimicrobial susceptibility testing according to CLSI standards.

The $bla_{\rm NDM-1}$ gene was transferred by biparental conjugation to either sodium azide-resistant *E. coli* J53 or *A. baumannii* ATCC 19606 from M15373 but not from M15274, conferring non-susceptibility to carbapenems on the recipient strain (Table 1). S1 nuclease digestion showed that both *A. pittii* and the transconjugant strains harboured a single plasmid of \sim 54 kb that hybridized with the $bla_{\rm NDM}$ probe. Plasmids gave negative results for all the Inc groups when assessed by PCR replicon typing. ¹²

Additionally, further characterization of 23 contemporary carbapenem-resistant *Acinetobacter* spp. isolated in the same institution from November 2012 to March 2013 (clinical strains and patient swab samples) revealed a lack of MBL production and matched *A. baumannii* by MALDI-TOF.

A. pittii has recently been recognized as a key organism for the dissemination of NDM, since it has been associated with the dispersal of this carbapenemase in such diverse scenarios as food of animal origin and sewage, and has been responsible for both sporadic human infection and large outbreaks in hospital units. 10,13,14 Until now, non-baumannii Acinetobacter spp. expressing NDM have not been described in the American continent. These A. pittii clinical isolates are the first characterized NDM-1 producers from Paraguay. The origin of NDM-1-positive A. pittii in Paraguay remains unclear, since no history of travel to the suggested reservoirs of NDM was established for either patient. Our finding of bla_{NDM-1} on plasmids of identical 54 kb size in A. pittii strains with a heterogeneous clonal background suggests that

bla_{NDM-1} most likely spread by the transfer of plasmids in *A. pittii*. Recent published data suggest that *A. pittii* could act as a potential NDM reservoir for Enterobacteriaceae based on the ease of plasmid transfer to *E. coli*, ¹¹ as observed in one of the isolates reported here. Despite this ability, no further cases of NDM producers have so far been observed in the hospital.

In conclusion, the emergence of NDM in *A. pittii* constitutes a public health concern in Latin America, highlighting the relevance of an integrated surveillance of carbapenemase producers.

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Transparency declarations

None to declare.

^bTransconjugant strains of *A. pittii* M15373.

^cMICs were determined using agar dilution; MICs of other antibiotics were determined using the Vitek 2C (AST-N082 card).

^dEDTA at a fixed concentration of 0.4 mM. The *bla_{VIM-11}*-producing *Pseudomonas aeruginosa* M5109 was used for quality control purposes.

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