

3 months before R178, on the same farm, indicating the animal carriage of the gene, integron and plasmid (data not shown). Also recently, *Acinetobacter baumannii* expressing OXA-23 isolated from cattle in France was reported.⁹ The occurrence of ESBLs in Enterobacteriaceae (*E. coli*, *Klebsiella* and *Salmonella*) isolated from food-producing animals and the increase in their prevalence is a current problem worldwide,^{6,10} but until now the presence of carbapenemases seemed to be mainly restricted to humans. Taking into account the low expression of some carbapenemases and their affinity for different carbapenems (i.e. intermediate resistance to ertapenem, and decreased susceptibility for imipenem and meropenem) in some isolates,^{2,3} the prevalence of carbapenemases in bacteria from livestock can be underestimated. Their level of expression could vary *in vivo*,³ depending on the selective pressure (i.e. frequent use of carbapenems in hospital settings). In fact, in Germany carbapenems are not allowed for the treatment of livestock animals. The presence of carbapenemase-encoding genes located on highly effective mobile genetic elements in the livestock environment, and the possibility of their transmission via food in the community and/or hospitals is worrying and an important issue for public health.

Acknowledgements

We thank B. Baumann and W. Barownick (BfR), and M. Thieck and H. Jansen (FU) for their technical support. We thank C. von Salviati and H. Laube for sampling work. We also thank F. Aarestrup, H. Hasman, R. Hendriksen and A. Carattoli for control strains, and Y. Pfeifer for her advice.

Funding

This work was supported by the Federal Institute for Risk Assessment, BfR (BfR-46-001; 45-005) and the RESET Project (FKZ01K1013B; BMVL, German Federal Ministry for Education and Research).

Transparency declarations

None to declare.

References

- Grundmann H, Livermore DM, Giske CG *et al.* Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. *Euro Surveill* 2010; **15**: pii=19711.
- Miriagou V, Cornaglia G, Edelstein M *et al.* Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. *Clin Microbiol Infect* 2010; **16**: 112–22.
- Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011; **17**: 1791–8.
- Carattoli A. Resistance plasmid families in Enterobacteriaceae. *Antimicrob Agents Chemother* 2009; **53**: 2227–38.
- Tato M, Coque TM, Baquero F *et al.* Dispersal of carbapenemase *bla*_{VIM-1} gene associated with different Tn402 variants, mercury transposons, and conjugative plasmids in Enterobacteriaceae and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2010; **54**: 320–7.
- Rodríguez I, Barownick W, Helmuth R *et al.* Extended-spectrum β -lactamases and AmpC β -lactamases in ceftiofur-resistant *Salmonella*

enterica isolates from food and livestock obtained in Germany during 2003–07. *J Antimicrob Chemother* 2009; **64**: 301–9.

7 Dallenne C, Da Costa A, Decré D *et al.* Development of a set of multiplex PCR assays for the detection of genes encoding important β -lactamases in Enterobacteriaceae. *J Antimicrob Chemother* 2010; **65**: 490–5.

8 Poirel L, Naas T, Nicolas D *et al.* Characterization of VIM-2, a carbapenem-hydrolyzing metallo- β -lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother* 2000; **44**: 891–7.

9 Poirel L, Berçot B, Millemann Y *et al.* Carbapenemase-producing *Acinetobacter* spp. in cattle, France. *Emerg Infect Dis* 2012; **18**: 523–5.

10 EFSA Panel on Biological Hazards (BIOHAZ). Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β -lactamases and/or AmpC β -lactamases in food and food-producing animals. *EFSA Journal* 2011; **9**: 2322.

J Antimicrob Chemother 2012

doi:10.1093/jac/dks101

Advance Access publication 29 March 2012

Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala

Fernando Pasteran¹, Ezequiel Albornoz¹,
Diego Faccone¹, Sonia Gomez¹, Claudia Valenzuela²,
Melissa Morales², Pavela Estrada³, Laura Valenzuela⁴,
Jorge Matheu⁵, Leonor Guerrero¹, Enrique Arbizú²,
Yeraldine Calderón², Pilar Ramon-Pardo⁵,
and Alejandra Corso^{1*}

¹Servicio Antimicrobianos, Instituto Nacional de Enfermedades Infecciosas (INEI)-ANLIS 'Dr. Carlos G. Malbrán', Ciudad Autónoma de Buenos Aires, Argentina; ²Sección Bacteriología, UCREVE/Laboratorio Nacional de Salud, Ciudad de Guatemala, Guatemala; ³Hospital Infantil de Infectología y Rehabilitación, Ciudad de Guatemala, Guatemala; ⁴Hospital General San Juan de Dios, Ciudad de Guatemala, Guatemala; ⁵Alert and Response and Epidemic Diseases, Pan American Health Organization/World Health Organization, Washington, DC, USA

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

Keywords: carbapenemases, multidrug resistance, outbreak

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.¹ Subsequently, isolates harbouring NDM have been mainly found in the Indian subcontinent, the Balkans and the UK, but also have been reported from many different countries in Asia, Europe, Africa, Oceania and North America.² However, NDM producers

Table 1. Antimicrobial susceptibility (MICs in mg/L) of NDM-producing *K. pneumoniae* clinical isolates and *E. coli* transconjugant and recipient strains

Antimicrobials	Clinical isolates		Transconjugant and recipient strains		
	<i>K. pneumoniae</i> N83 (M13717)	<i>K. pneumoniae</i> N162 (M13716)	<i>E. coli</i> M13765 ^a	<i>E. coli</i> M13766 ^a	<i>E. coli</i> J53
Imipenem	4	4	8	4	≤0.5
Meropenem	4	4	2	2	≤0.5
Ertapenem	>4	>4	≤2	4	≤2
Cefoxitin	>16	>16	>16	>16	≤8
Piperacillin/tazobactam	>64	>64	>64	>64	≤4
Third-generation cephalosporins ^b	>32	>32	>32	>32	≤1
Cefepime	>16	>16	8	8	≤8
Aztreonam	>16	>16	>16	>16	≤8
Gentamicin	8	8	≤4	≤4	≤4
Amikacin	≤8	≤8	≤8	≤8	≤8
Nalidixic acid	≤16	≤16	≤16	≤16	≤16
Levofloxacin	1	1	≤0.12	≤0.12	≤0.12
Ciprofloxacin	2	2	≤0.06	≤0.06	≤0.06
Trimethoprim/sulfamethoxazole	>2	>2	≤2	≤2	≤2
Minocycline	>8	>8	≤4	≤4	≤4
Tigecycline	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5
Chloramphenicol	>16	>16	≤8	≤8	≤8
Nitrofurantoin	64	64	≤32	≤32	≤32
Fosfomycin	≤16	≤16	≤16	≤16	≤16
Colistin	≤1	≤1	≤1	≤1	≤1

^a*E. coli* M13765 and M13766 are transconjugant strains of *K. pneumoniae* N83 (M13717) and N162 (M13716) isolates, respectively.

^bThird-generation cephalosporins included ceftazidime and cefotaxime.

have not been reported yet in Latin America. Here, we report two NDM-1-producing *K. pneumoniae* isolates identified in Guatemala.

Since 1996, the Pan American Health Organization (PAHO) has supported a regional surveillance system, the Latin American Network for the Surveillance of Antimicrobial Resistance (ReLAVRA), which is based on routine laboratory data and strengthening the laboratory capability through the training of laboratory personnel and integrated by 794 laboratories, including 21 national reference laboratories.^{3,4} In June 2010, a regional protocol for the detection of carbapenemases was harmonized and implemented through ReLAVRA.⁵ Briefly, metallo-β-lactamase (MBL) production is suspected in isolates that exhibit: (i) imipenem inhibition zones ≤22 mm or a Vitek 2C MIC of imipenem ≥2 mg/L plus a meropenem MIC ≥1.0 mg/L;⁶ and (ii) a positive synergy test result between carbapenems and EDTA discs.

From January 2011 to February 2011, following the ReLAVRA algorithm, the Health National Laboratory from Guatemala confirmed an MBL phenotype in two *K. pneumoniae* isolates. This phenotype had not previously been observed in Enterobacteriaceae from Guatemala. The first case corresponded to a 1-year-old patient with nosocomial pneumonia and septic shock referred in January 2011 to a tertiary paediatric referral hospital because of a lack of response to meropenem plus vancomycin treatment (14 days). *K. pneumoniae* N83 (M13717) was recovered from a catheter. Vancomycin treatment was discontinued and piperacillin/tazobactam plus amikacin was added. After 14 days of treatment the patient was discharged alive.

The second case corresponded to an adult patient admitted in February 2011 to a tertiary adult referral hospital because of head and neck trauma from gunfire. *K. pneumoniae* N162 (M13716) was recovered from tracheal secretions. Six days after admission, the patient's condition worsened and the patient expired, probably related to the multiple traumatic injuries.

The strains were submitted to the regional reference laboratory (Servicio Antimicrobianos, INEI-ANLIS 'Dr. Carlos G. Malbrán') for further characterization. Antimicrobial drug susceptibility testing using Sensititre panels (Trek Diagnostic Systems, Cleveland, OH, USA) revealed identical resistance profiles for both *K. pneumoniae* isolates (Table 1). The strains were resistant to all the β-lactams tested, trimethoprim/sulfamethoxazole and minocycline, and displayed intermediate susceptibility to ciprofloxacin, gentamicin and chloramphenicol.⁷ They remained susceptible to amikacin, nalidixic acid, levofloxacin⁷ and, according to EUCAST standards, to tigecycline, colistin and fosfomycin. Both isolates tested positive in the modified Hodge test and MBL production was confirmed by using a combination disc test.⁸

In both isolates, PCR screening followed by DNA sequencing detected the presence of *bla*_{NDM-1} [the primers used were NDM-F (5'-CTATTACTAGGCCTCGCATT-3') and NDM-R (5'-ATAA AACGCCTCTGTACAT-3')], *bla*_{CTX-M-15}, *bla*_{SHV-11}, *bla*_{SHV-12}, *bla*_{TEM-1} and *bla*_{OXA-1}, as well other genes affecting quinolone activity, specifically *qnrB1* and *aac(6')-Ib-cr*. Amplification for other genes, such as *bla*_{CMY}, *aac(6')-Ib*, *bla*_{VIM}, *bla*_{SPM}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{VEB} and *bla*_{GES}, was negative.

PFGE analysis showed a similar profile for both isolates (two bands of difference). The clonal relationship was further assessed by multilocus sequence typing, which showed that both *K. pneumoniae* isolates belonged to sequence type (ST) 17 (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>). Notably, it does not correspond to the most common STs, ST14 and ST147, identified in NDM-1-positive *K. pneumoniae*.² ST17 (clonal complex 17) appears to be widespread independently of *bla*_{NDM}. Most ST17 isolates in the database produce *bla*_{CTX-M-15}, as observed in these strains from Guatemala.

Transconjugants from both clinical isolates were obtained at 28 and 35°C, with selection based on cefoxitin (10 mg/L) and azide (200 mg/L), and using *E. coli* J53 as the recipient strain. Transconjugants exhibited resistance to all β-lactams, including aztreonam, indicating successful co-transfer of *bla*_{SHV-12} along with *bla*_{NDM-1} that was further confirmed by PCR and DNA sequencing. The transfer of NDM-1 was associated with plasmids that gave negative results for all the Inc groups when assessed by PCR replicon typing.⁹

In conclusion, these *K. pneumoniae* clinical isolates are the first characterized NDM-1-producing Enterobacteriaceae from Latin America. Additionally, these isolates represent the first isolates with a novel combination of resistance genes, such as *bla*_{SHV-11} plus *bla*_{SHV-12} and *aac(6′)-Ib-cr* plus *qnrB1*. A recent history of contact or travel to the suggested reservoirs of NDM was not established for both patients. Since both *K. pneumoniae* strains analysed in this study belonged to the same clonal type and epidemiological links between the two cases were not apparent, we can speculate that this clone had already spread silently in Guatemala City. Further studies are being conducted in order to evaluate the putative origin of this clone. Given this situation, in November 2011, PAHO issued a regional alert, to strengthen the Latin American surveillance of carbapenemase producers and to highlight the importance of microbiological detection of NDM carbapenemase.¹⁰

Acknowledgements

We are in debt to Omar Veliz from the Servicio Antimicrobianos, INEI-ANLIS ‘Dr. Carlos G. Malbrán’, Ricardo Mena from the Hospital General San Juan de Dios and H. Divas from the Hospital Infantil de Infectología y Rehabilitación for their valuable contribution, and to Medica-Tec Argentina for providing Sensititre panels.

Funding

This study was supported, in part, by a grant provided by the Spanish Agency of International Cooperation and Development/Agencia Española de Cooperación Internacional para el Desarrollo (AECID) to the Pan American Health Organization.

Transparency declarations

None to declare.

References

1 Yong D, Toleman MA, Giske CG *et al.* Characterization of a new metallo-β-lactamase gene, *bla*_{NDM-1}, and a novel erythromycin esterase

gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother* 2009; **53**: 5046–54.

2 Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011; **17**: 1791–8.

3 Schmunis G, Salvatierra-Gonzalez S. Birth of a public surveillance system: PAHO combats the spread of antimicrobial resistance in Latin America. *The APUA Newsletter* 2006; **24**: 6–8.

4 OPS/PAHO. *Informe Anual de la Red Latinoamericana de Vigilancia de la Resistencia a los Antimicrobianos, 2009*. http://new.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=14877&Itemid=4108 (16 February 2012, date last accessed).

5 Corso A, Guerriero L, Pasterán F *et al.* Capacidad de los laboratorios nacionales de referencia en Latinoamérica para detectar mecanismos de resistencia emergentes. *Rev Panam Salud Publica* 2011; **30**: 619–26.

6 Pasteran F, Lucero C, Soloaga R *et al.* Can we use imipenem and meropenem Vitek 2 MICs for detection of suspected KPC and other carbapenemase producers among species of Enterobacteriaceae? *J Clin Microbiol* 2011; **49**: 697–701.

7 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement M100-S22*. CLSI, Wayne, PA, USA, 2012.

8 Pasteran F, Veliz O, Guerriero L *et al.* A combination disk tests (CDT) for the detection of KPC and MBL with the use of meropenem (MEM) disks supplemented with 3-aminophenylboronic (APB), EDTA or high-load cloxacillin (CLO) for simultaneous use in Enterobacteriaceae (ENT) and *Pseudomonas aeruginosa* (PAE). In: *Abstracts of the Fifty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2011*. Abstract D-679. American Society for Microbiology, Washington, DC, USA.

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

10 OPS/PAHO. *Carbapenemases of Type New Delhi Metallo-β-lactamase (NDM)*. http://new.paho.org/hq/index.php?option=com_content&task=view&id=6222&Itemid=2291 (13 February 2012, date last accessed).

J Antimicrob Chemother 2012

doi:10.1093/jac/dks100

Advance Access publication 28 March 2012

VIM-2 metallo-β-lactamase-producing *Pseudomonas aeruginosa* causing an outbreak in South Africa

Rachael Kiera Jacobson^{1–3*}, Nadia Minenza¹, Mark Nicol^{1–3} and Colleen Bamford^{1–3}

¹Division of Medical Microbiology, University of Cape Town, Anzio Road, Observatory, 7925, Cape Town, South Africa; ²National Health Laboratory Service, Groote Schuur Hospital, Anzio Road, Observatory, 7925, Cape Town, South Africa; ³NICD Unit for Molecular Epidemiology, Groote Schuur Hospital, Anzio Road, Observatory, 7925, Cape Town, South Africa

*Corresponding author. Tel: +27-21-4044476; Fax: +27-21-4044472; E-mail: rachaeljacobson@gmail.com