

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.

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Transparency declarations

None to declare.

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

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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.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 as 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

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Transparency declarations

None to declare.

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

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