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 both K. pneumoniae isolates belonged to sequence type (ST) 17 (http://www.pasteur.fr/recherche/genopole/PG/PB/Kpneumoniae.html). Notably, it does not correspond to the most common STs, ST14 and ST147, identified in NDM-1-positive K. pneumoniae. ST17 (clonal complex 17) appears to be widespread independently of blaNDM. Most ST17 isolates in the database produce blaCTX-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 blaSHV-12 along with blaNDM-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 blaSHV-11 plus blaSHV-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

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

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Sir,

_Pseudomonas aeruginosa_ is an opportunistic pathogen that has been implicated in nosocomial outbreaks in immunocompromised patients worldwide. We present an outbreak caused by a multidrug-resistant _P. aeruginosa_ (MRPA) clone in a haematology unit of a tertiary academic hospital in Cape Town, South Africa. Fifteen MRPA isolates were recovered from separate patients between January 2010 and April 2011, including 10 from blood, 2 from stool, 1 from bile, 1 from urine and 1 from a catheter tip. The majority of the patients were severely neutropenic following stem cell transplants and eight of them died.

The genetic relatedness of the isolates was investigated using PFGE according to a previously published protocol with minor amendments. Whole genomic DNA was digested in situ with SpeI (New England Biolabs, Inc., UK) and the resulting restriction fragments were separated on a 1% agarose gel using a CHEF-DRII GeneNavigator apparatus (GE Healthcare, Piscataway, NJ, USA). Restriction profiles were analysed using GelCompar II version 5.1 (Applied Maths, St-Martens-Latem, Belgium). A dendrogram indicating the levels of similarity between the isolates was created using the Dice similarity coefficient. The band tolerance and optimizations were set at 1%, and a similarity threshold of 80% was used to define clusters.

The profiles of 10 of the 15 isolates were indistinguishable and were assigned to cluster A. One isolate, with 77% similarity to cluster A, was assigned subtype A1. The remaining four isolates were all unique. To further characterize the genetic background of this MRPA clone, multilocus sequence typing (MLST) of three representative isolates from cluster A, spanning the defined outbreak period, were selected. MLST indicated that these three isolates belong to sequence type (ST) 233.

Identification and susceptibility testing was performed on Vitek 2 (bioMérieux, Marcy l’Etoile, France). Imipenem and meropenem MICS were determined by Etests, according to the manufacturer’s specification (bioMérieux). Fourteen of the isolates expressed high levels of resistance to imipenem (MIC $\geq$128 mg/L) and meropenem (MIC $\geq$128 mg/L). The 15th strain was susceptible to both imipenem (MIC $\leq$1 mg/L) and meropenem (MIC $\leq$2 mg/L).

As metallo-β-lactamasess (MBLs) are considered a major mechanism of carbapenem resistance in _P. aeruginosa_, PCR assays were carried out using primers for the detection of the MBL-encoding genes _bla_ (IMPF 5′-ATTGACACTCCATTAC-3′/IMPR 5′-AACAACAGTGTGCG-3′) and _bla_ (VIMF 5′-GTGAGTATCCGACAGTGC-3′/VIMR 5′-GAGCAAGTCTAGACCG-3′).

Although a PCR product of the expected size was obtained from a control _P. aeruginosa_ strain carrying _bla_ (gift from Y. Hirakata, Tohoku University Graduate School of Medicine, Japan), no products were obtained from the 15 Cape Town _P. aeruginosa_ isolates. However, amplicons of the expected size for the _bla_ gene were obtained for the 10 strains in PFGE cluster A and the closely related strain, A1, as well as the _bla_ Positive control (gift from P. Nordmann, Hôpital de Bicêtre, France). No PCR products were obtained from the four unrelated _P. aeruginosa_ isolates. PCR products from the 11 strains were purified (QIAquick® PCR purification kit, QIAGEN, Germany) and sequencing analysis revealed the _bla_ gene in all 11 strains. Additional PCR screening for the NDM, SPM, KPC and GES β-lactamase genes for all 15 isolates revealed that the single isolate, sensitive to both imipenem and meropenem and cultured from a catheter tip, carried a _bla_ gene, which has previously been implicated in a South African outbreak. MLST typing indicated that this carbapenem-susceptible strain belonged to ST625, a new genotype not previously described.

Relatively little is known regarding the prevalence of carbapenem-hydrolysing enzymes in African countries. This outbreak of _MBL_ carrying _P. aeruginosa_ highlights the urgent need for the development of more active surveillance systems in South Africa and the importance of molecular epidemiology in a hospital outbreak situation.

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