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Emergence of NDM-1-producing Acinetobacter baumannii in Belgium

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Sir,

New Delhi metallo-β-lactamase (NDM) is a class B β-lactamase that confers resistance to virtually all β-lactams, including carbapenems. Initially reported in Sweden in Enterobacteriaceae isolates from a patient transferred from India, NDM-1 is currently spreading worldwide, including Belgium, especially in Enterobacteriaceae.1 Recently the presence of blaNDM-1 was also reported in Acinetobacter baumannii isolates from India, China and Germany, suggesting a broad host range distribution of this gene.1–4 Here, we report an NDM-1-producing A. baumannii detected in Belgium that was isolated from a patient following medical repatriation from Algeria.

In early 2011, a young male patient was admitted to the emergency ward of an Algerian hospital for severe cranial and thoracic trauma following a road traffic accident. He presented with a Glasgow Coma Score (GCS) of 07/15 and was rapidly intubated, ventilated and transferred to the intensive care unit (ICU).

While hospitalized in Algeria, he experienced three successive, distinct episodes of bloodstream infection, due to Acinetobacter spp., Candida albicans and Klebsiella pneumoniae, but no clinical information was available concerning the possible sources of these infections. In August, the patient was transferred, in a vegetative state (GCS of 02/15), to the ICU of a Belgian hospital. He was immediately placed in a single room with contact isolation precautions.

Screening for asymptomatic intestinal carriage of carbapenemase producers was carried out upon admission, by rectal swabbing cultured by direct plating on ChromID™ ESBL Agar (bioMérieux, Marcy l’Étoile, France). The recovered isolate was identified as A. baumannii (Ab 11314) by MicroFlex LT (Bruker Daltonik, Bremen, Germany). By disc diffusion, Ab 11314 was found to be multiresistant and synergy was observed in a double disc test utilising imipenem and EDTA (420 µg), suggesting involvement of a metallo-β-lactamase in resistance to β-lactams. Use of the CLSI microdilution method confirmed that the isolate was resistant to β-lactams including ceftazidime (MIC >64 mg/L), cefepime (MIC >64 mg/L), imipenem (MIC 4 mg/L; Etest MIC >32 mg/L), meropenem (MIC 4 mg/L; Etest MIC >32 mg/L), amikacin (MIC >32 mg/L) and ciprofloxacin (MIC >32 mg/L), and susceptible to tigecycline (MIC 0.125 mg/L), colistin (MIC 0.5 mg/L) and minocycline (MIC <2 mg/L).

Analysis of β-lactamase-coding genes by an MDR CT102 array (Check-Points, Wageningen, The Netherlands) and by PCR sequencing targeting metallo-β-lactamases and OXA-carbapenemases revealed the presence of blaNDM-1. Plasmid extraction was performed with a Qiagen plasmid kit, and this revealed a single 174 kb untypable plasmid.5 This plasmid was efficiently electroporated and transferred by mating experiments to the A. baumannii recipient strain N9040364, and it conferred resistance to aminoglycosides (gentamicin, tobramycin and amikacin) only (data not shown). The blaNDM-1 gene was not associated with this plasmid suggesting that it was located in the chromosome. PCR mapping and sequencing revealed that blaNDM-1 was located between two direct repeats of the IS6052 element in a transposon similar to the one in A. baumannii Ab 161/07 reported from Germany (accession no. HQ857107).5 Nevertheless, a PCR experiment performed with Ab 161/07 as a positive control revealed that in Ab 11314, in contrast, the mfs gene (a shikimate-transporter-coding gene belonging to the major facilitator superfamily) was not disrupted, indicating that the ISAb125 transposon was inserted elsewhere in the chromosome. By multilocus sequence typing (MLST) (http://pubmlst.org/abaumannii/), A. baumannii Ab 11314 was shown to belong to sequence type (ST) 92 (allelic profile: 1-3-3-2-2-7-3), the founding genotype of clonal complex (CC) 92, which includes European clone 2 (EU2) and worldwide lineage 2 (WW2). CC92 is one of the most prevalent CCs worldwide, mostly represented by OXA-23-producing strains. Sequencing analysis of the intrinsic blaOXA-51-like and blaADC genes showed that this strain harboured blaOXA-64 and blaOXA-26, neither of which was downstream of ISAb1 or ISAb125. These two intrinsic genes were identical to those recovered in the NDM-1-expressing A. baumannii Ab 161/07, but nevertheless presented a very different MLST profile (1-15-2-28-1-1 new allele-32), not related to any CC described up to now.

This clinical isolate is the first characterized NDM-1-producing A. baumannii from Belgium. As yet, there have been only a few reports of NDM-producing A. baumannii, and the only case reported in Europe was from Germany in a patient transferred from Serbia in 2007.7 In the present case, the NDM-1-producing A. baumannii isolate originated from North Africa (Algeria) where NDM-1 has not yet been described in A. baumannii, although another single case of A. baumannii producing an NDM-1 variant (NDM-2) was recently reported from Egypt.8 This brief report further highlights the wide distribution of NDM-producing organisms around the world, their constant evolving spread.
and underlines the importance of systematic screening upon admission and early application of strict barrier precautions for any transferred patient, whatever the country of origin.

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Transparency declarations

None to declare.

References

8 In mid-2011, the stool sample of a 1-year-old infant was found to have carbapenem-resistant Enterobacteriaceae (CRE) upon admission screening. The infant was admitted because of cough and intermittent fever in the preceding 2 weeks. The family had travelled to and stayed in Hunan Province, China in the preceding month. Following onset of the symptoms, the infant had been admitted to a hospital in Hunan for 3 days. The patient was given a diagnosis of bronchitis and had been treated with a course of intravenous cefoperazone. At presentation to our hospital, the patient had a fever of 38°C, but the chest examination was unremarkable. The patient was treated conservatively and the fever resolved without further antibiotic treatment. The patient was discharged 2 days later. In accordance with the screening policy, stool samples were obtained for surveillance culture.

In brief, a red-bean-size faecal pellet was suspended in saline. A 10 μL aliquot of the suspension was then removed and plated directly onto a ChromID ESBL plate (bioMérieux, Marcy l’Etoile, France). In addition, a broth enrichment step was performed by inoculating another 10 μL aliquot of the faecal suspension into nutrient broth with 1 mg/L meropenem. All plates were incubated at 37°C overnight and then subcultured onto a MacConkey agar plate with 1 mg/L meropenem. All isolates were incubated at 37°C in air for 20 h. All distinct colony types recovered from either the chromogenic or the MacConkey media were investigated for evidence of carbapenemase activity, using the combined disc method and boronic acid or EDTA as inhibitor. All isolates were identified using VITEK 2 (bioMérieux), and antimicrobial susceptibility was determined using the CLSI disc diffusion method.

After the patient’s stool samples were found to carry CRE, stool samples from the infant’s parents and other family members were also cultured using the same methodology. A total of four CRE isolates were recovered from the faecal samples of the child and her mother (Table 1). Cultures of the faecal samples from the other household members (father, grandfather, grandmother and aunt) were negative. All four isolates (two Escherichia coli, one Klebsiella pneumoniae and one Enterobacter aerogenes) exhibited synergy with EDTA in the combined disc testing. No synergy with boronic acid was observed. PCR and sequencing, using previously described methods, confirmed the presence of NDM-1 in the four CRE isolates. The presence of additional β-lactamase genes, including other metallo-β-lactamase (IMP, VIM, GIM, SPM and SIM), CTX-M and OXA-48-like genes, was also tested by PCR and sequencing. This allowed identification of additional extended-spectrum β-lactamase (ESBL) genes in the K. pneumoniae isolate.