First Report of NDM-1-Producing Acinetobacter baumannii Sequence Type 25 in Brazil

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New Delhi metallo-β-lactamase 1 (NDM-1) was first identified in Brazil in Enterobacter hormaechei and Providencia rettgeri in 2013. Here, we describe the first case of NDM-1-producing Acinetobacter baumannii sequence type 25 isolated from the urinary tract of a 71-year-old man who died of multiple complications, including A. baumannii infection. The NDM-1 gene was detected by quantitative PCR, and its sequence confirmed its presence in an ~100-kb plasmid.

Since the first description of New Delhi metallo-β-lactamase (NDM) in 2008 in a Swedish patient who had traveled to India (1), many other countries have reported this resistance mechanism; by 2010, it was isolated in almost all continents and nearly 40 countries (2–6). Most cases are directly linked to India, Pakistan, Bangladesh, or the Balkans region (4, 6, 7). In Latin America, it was first described in 2011 in Klebsiella pneumoniae from Guatemala and Colombia (8, 9). NDM was detected in other South American countries in 2012. NDM-1 was first described in Brazil in 2013, in Providencia rettgeri (10) and Enterobacter hormaechei (11) isolated from the same hospital. Here, we describe the first case of NDM-1-producing Acinetobacter baumannii in Brazil.

A 71-year-old man was admitted to the intensive care unit at a 117-bed hospital in Londrina, State of Paraná, Southern Brazil, on 5 December 2013. He had chronic obstructive pulmonary disease and was hospitalized for respiratory failure. On day 42 (15 January 2014), because infectious disease was suspected, urine and blood samples were collected. Empirical antibiotic therapy of intravenous imipenem and vancomycin was initiated. Carbapenem-resistant A. baumannii (CRAB) was isolated from the urine sample, with a colony count above 10^5 CFU/ml, and vancomycin-resistant enterococci (VRE) were recovered from blood culture. On day 51, the patient’s clinical condition worsened, and he was intubated. The antimicrobial therapy was changed to polymyxin and amikacin on day 54. The CRAB isolate was sent to a reference laboratory on day 56 for molecular detection of resistance genes. The patient had no history of overseas travel. Two additional multidrug-resistant bacteria were also isolated from the respiratory tract (one CRAB on day 51 and one carbapenem-resistant Pseudomonas aeruginosa on day 53). These isolates were blaNDM negative, but there was an ongoing CRAB outbreak in the institution, and a VRE outbreak had occurred 3 weeks before. The patient died on day 60 of hospitalization.

Automated tests (Vitek 2; bioMérieux), 16S rRNA gene sequencing (Microseq 500; Life Technologies), and PCR for blaOXA-51-like genes confirmed identification of the first CRAB isolate. Common OXA-like carbapenemases in A. baumannii (blaOXA-24-like, blaOXA-23-like, and blaOXA-143) were not detected by multiplex PCR (12). Screening for NDM using an EDTA inhibition disc method adapted to A. baumannii as previously described (13) showed a 14–18-mm increase on carbapenem discs with EDTA compared to discs without EDTA. An EDTA inhibition test was also positive using Etest MBL strips (imipenem MIC of >256 mg/liter; imipenem and EDTA MIC of ≤1 mg/liter). The blaNDM-1 gene was detected by multiplex quantitative PCR (qPCR) for Klebsiella pneumoniae carbapenemase (KPC) and NDM following a protocol from the Centers for Disease Control and Prevention (CDC) (14) in a 7300 real-time PCR system (Life Technologies, Foster City, CA, USA) and by an automated Multiplex qPCR using BD-MAX equipment and a commercial carbapenem-resistant Enterobacteriaceae (CRE) assay (Becton and Dickinson). NDM was also confirmed by sequencing (15). Susceptibility tests were conducted using the disk diffusion method, automated testing (Vitek 2), and Etest (bioMérieux). NDM-1-positive A. baumannii was resistant to meropenem, imipenem, all cephalosporins (including cefepime), aztreonam, amikacin, gentamicin, tobramycin, doxycycline, minocycline, tetracycline, and trimethoprim-sulfamethoxazole. Although there is no CLSI breakpoint for tigecycline in A. baumannii, the MIC determined by Etest was 3 mg/liter (resistant considering Enterobacteriaceae breakpoints). The only antimicrobial drug effective against the isolate was colistin (MIC of 1 mg/liter). Additional screening of the blaNDM gene was performed by PCR and sequencing using primers previously described (16), which aligned at base pair positions 3 to 20 and 776 to 790 of the gene. The reaction yielded a 787-bp-sized amplicon of the blaNDM gene, and its sequencing allowed us to confirm the NDM-1 allele. Southern blot analysis (17) showed the blaNDM-1 gene in an ~100-kb plasmid, and mul-

tiplex sequence typing (MLST) analysis based on the Pasteur Institute scheme (http://www.pasteur.fr) showed that this isolate belonged to sequence type 25 (ST25) clonal complex 25. Although this is not a prevalent clonal complex in Brazil, this ST has been detected in other Brazilian states and is associated with OXA-23 carbapenemase production (18). A. baumannii ST25 has been de-

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scribed in two different isolates from countries in the Balkans region and one isolate from Africa (7, 19).

Screening and molecular analysis of >100 Enterobacteriaceae and A. baumannii isolates at the same hospital from January to July 2014 revealed no additional NDM-1-positive bacteria.

Carbapenemases are a growing resistance problem worldwide (5, 20). CRAB strains are globally distributed, particularly in Brazil, where OXA-23 outbreaks have been described since 1999 (21–23) and have been shown to survive in hospital environments (24). KPC and NDM-1 can hydrolyze most β-lactam antibiotics, leaving usually only colistin, tigecycline, and fosfomycin as therapeutic options (5). Although most NDM genes are found in Enterobacteriaceae (4), they have also been detected in Acinetobacter species (5, 7, 19, 25). The first case of NDM in Acinetobacter was reported in India in 2010 (26), although the first strain, isolated in 2007, was linked to the Balkans and was chromosomally encoded. Other reports from China, Egypt, and France followed (5–7, 27). The Latin American countries Honduras (28) and Paraguay (29) have also reported Acinetobacter pittii isolates. ST25 NDM-1-positive A. baumannii originating from the Balkan region has been reported in European studies (13, 25). We could not trace the origin of the blaNDM gene in the present case, since there was no history of overseas travel, and to our knowledge, this is the first reported NDM isolate in Paraná State. Although NDM-1-positive Enterobacteriaceae have been isolated in Rio Grande do Sul (10, 11), approximately 1,200 km south of Londrina, there was no history of patient transfer between these locations. Recently, NDM-positive A. pittii was isolated in Rio Grande do Sul (A. Barth, personal communication) and in the bordering country of Paraguay (29), but there is no apparent epidemiological link. Further investigations are under way to establish a hypothesis for the origin of this blaNDM-1 plasmid.

Bonin et al. recently suggested that A. baumannii not only might accept resistance genes but also could act as a gene donor, spreading resistance genes to other bacteria, including Enterobacteriaceae (27). This possibility emphasizes our concerns about dissemination of these genes in our country as in many other countries where NDM-1-producing A. baumannii have been isolated, making these findings a global public health matter, as suggested by Johnson and Woodford (6).

Strict control and prevention measures should be taken, once NDM-1-positive A. baumannii have been identified, to prevent transfer of this resistance gene to Enterobacteriaceae.

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