The first report of NDM-1-producing *Acinetobacter baumannii* ST 25 in Brazil

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Running title: First NDM-1 *Acinetobacter baumannii* in Brazil

New-Delhi metallo-beta-lactamase (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
(ST) 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 polymerase chain reaction, the sequence of which
was confirmed its presence in a ~100 kb plasmid

Since the first description of New-Delhi metallo-beta-lactamase (NDM)
in 2008 in a Swedish patient who had traveled to India, (1) many other
countries have reported this resistance: 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 *A. baumannii* in
Brazil.

A 71-year-old man was admitted to the ICU at a 117-bed hospital in
Londrina, State of Paraná, Southern Brazil, on December 5, 2013. He had
chronic obstructive pulmonary disease and was hospitalized for respiratory
failure. On day 42 (January 15, 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 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 multi-drug-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 *bla*$_{NDM}$-negative, but there was an ongoing CRAB outbreak in the institution and a VRE outbreak had occurred three weeks before. The patient died on day 60 of hospitalization.

Automated tests (Vitek 2®, Biomerieux), 16S rDNA sequencing (Microseq 500®, Life Technologies), and PCR for *bla*$_{OXA-51}$-like genes confirmed identification of the first CRAB isolate. Common OXA-like carbapenemases in *A. baumannii* (*bla*$_{OXA-23}$-like, *bla*$_{OXA-24}$-like, *bla*$_{OXA-58}$-like, and *bla*$_{OXA-143}$) 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 minimum inhibitory concentration [MIC] > 256 μg/L; imipenem plus EDTA MIC ≤ 1 μg/L). The *bla*$_{NDM-1}$ gene was detected by multiplex qPCR for *Klebsiella pneumoniae* carbapenemase (KPC) and NDM following a protocol from the Centers for Disease Control (CDC) (14) in a 7300 Real Time PCR System® (Life Technologies, Foster City, CA, USA) and by an automated-Multiplex qPCR reaction 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 E-test® (Biomerieux). 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*, MIC determined by E-test was 3 mg/L (resistant considering *Enterobacteriaceae* breakpoints). The only antimicrobial drug effective against the isolate was colistin (MIC: 1 mg/L). Additional screening of *bla*<sub>NDM</sub> gene was performed by PCR and sequencing using primers previously described (16) which aligned at position 3-20bp and 776-790bp respectively of the gene. The reaction yielded a 787bp-sized amplicon of the *bla*<sub>NDM</sub> gene and its sequencing allowed us to confirm NDM-1 allele. Southern blot analysis (17) showed the *bla*<sub>NDM-1</sub> gene in a ~100 kb plasmid and multilocus sequence typing (MLST) analysis based on the Pasteur Institute scheme (http://www.pasteur.fr) showed that this isolate belonged to sequence type (ST) 25-Clonal Complex 25. Although this 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). ST 25 *A. baumannii* has been described 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 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 A. pittii isolates. ST-25 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 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 (Dr. A. Barth-personal communication) and in the bordering country of Paraguay (29), but there is no apparent epidemiological link. Further investigations are underway to establish a hypothesis for the origin of this blaNDM plasmid.

Bonnin et al. recently suggested that A. baumannii might not only accept resistance genes, but could also 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 resistance genes to other Enterobacteriaceae.

References:


