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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 bla_{NDM} 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 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- to 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 bla_{NDM-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 bla_{NDM} 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 bla_{NDM} gene, and its sequencing allowed us to confirm the NDM-1 allele. Southern blot analysis (17) showed the bla_{NDM-1} gene in an ~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 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 bla_{NDM} 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 bla_{NDM-1} plasmid.

Bonnin 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*.

REFERENCES

1. **Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR.** 2009. Characterization of a new metallo-beta-lactamase gene, *bla*(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. **53:**5046–5054. http://dx.doi.org/10.1128/AAC.00774-09.
2. **Mochon AB, Garner OB, Hindler JA, Krogstad P, Ward KW, Lewinski MA, Rasheed JK, Anderson KF, Limbago BM, Humphries RM.** 2011. New Delhi metallo-β-lactamase (NDM-1)-producing *Klebsiella pneumoniae*: case report and laboratory detection strategies. J. Clin. Microbiol. **49:**1667–1670. http://dx.doi.org/10.1128/JCM.00183-11.
3. **Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE.** 2011. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY antimicrobial surveillance program, 2006–2007. Antimicrob. Agents Chemother. **55:**1274–1278. http://dx.doi.org/10.1128/AAC.01497-10.
4. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of Carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **17:**1791–1798. http://dx.doi.org/10.3201/eid1710.110655.
5. **Dortet L, Poirel L, Nordmann P.** 2014. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. Biomed. Res. Int. **2014:**1–12. http://dx.doi.org/10.1155/2014/249856.
6. **Johnson AP, Woodford N.** 2013. Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. J. Med. Microbiol. **62:**499–513. http://dx.doi.org/10.1099/jmm.0.052555-0.
7. **Bonnin RA, Poirel L, Naas T, Pirs M, Seme K, Schrenzel J, Nordmann P.** 2012. Dissemination of New Delhi metallo-β-lactamase-1-producing *Acinetobacter baumannii* in Europe. Clin. Microbiol. Infect. **18:**E362–E365. http://dx.doi.org/10.1111/j.1469-0691.2012.03928.x.
8. **Pasteran F, Albornoz E, Faccone D, Gomez S, Valenzuela C, Morales M, Estrada P, Valenzuela L, Matheu J, Guerriero L, Arbizú E, Calderón Y, Ramon-Pardo P, Corso A.** 2012. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Guatemala. J. Antimicrob. Chemother. **67:**1795–1797. http://dx.doi.org/10.1093/jac/dks101.
9. **Escobar Pérez JA, Olarte Escobar NM, Castro-Cardozo B, Valderrama Márquez IA, Garzón Aguilar MI, Martinez de la Barrera L, Barrero Barreto ER, Marquez-Ortiz RA, Moncada Guayazán MV, Vanegas Gómez N.** 2013. Outbreak of NDM-1-producing *Klebsiella pneumoniae* in a neonatal unit in Colombia. Antimicrob. Agents Chemother. **57:**1957–1960. http://dx.doi.org/10.1128/AAC.01447-12.
10. **Carvalho-Assef APD, Pereira PS, Albano RM, Berião GC, Chagas TPG, Timm LN, Da Silva RCF, Falci DR, Asensi MD.** 2013. Isolation of NDM-producing *Providencia rettgeri* in Brazil. J. Antimicrob. Chemother. **68:**2956–2957. http://dx.doi.org/10.1093/jac/dkt298.
11. **Carvalho-Assef APD, Pereira PS, Albano RM, Berião GC, Tavares CP, Chagas TPG, Marques EA, Timm LN, Da Silva RCF, Falci DR, Asensi MD.** 2014. Detection of NDM-1-, CTX-M-15-, and qnrB4-producing *Enterobacter hormaechei* isolates in Brazil. Antimicrob. Agents Chemother. **58:**2475–2476. http://dx.doi.org/10.1128/AAC.02804-13.
12. **Higgins PG, Lehmann M, Wisplinghoff H, Seifert H.** 2010. *gyrB* multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3. J. Clin. Microbiol. **48:**4592–4594. http://dx.doi.org/10.1128/JCM.01765-10.
13. **Bonnin RA, Naas T, Poirel L, Nordmann P.** 2012. Phenotypic, biochemical, and molecular techniques for detection of metallo-β-lactamase NDM in *Acinetobacter baumannii*. J. Clin. Microbiol. **50:**1419–1421. http://dx.doi.org/10.1128/JCM.06276-11.
14. **Centers for Disease Control and Prevention.** 2010. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1) genes. CDC, Atlanta, GA.
15. **Nordmann P, Poirel L, Carrër A, Toleman MA, Walsh TR.** 2011. How to detect NDM-1 producers. J. Clin. Microbiol. **49:**718–721. http://dx.doi.org/10.1128/JCM.01773-10.
16. **Sidjabat H, Nimmo GR, Walsh TR, Binotto E, Htin A, Hayashi Y, Li J, Nation RL, George N, Paterson DL.** 2011. Carbapenem resistance in *Klebsiella pneumoniae* due to the New Delhi metallo-β-lactamase. Clin. Infect. Dis. **52:**481–484. http://dx.doi.org/10.1093/cid/ciq178.
17. **Sambrook J, Russell DW.** 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
18. **Chagas TPG, Carvalho KR, de Oliveira Santos IC, Carvalho-Assef APD, Asensi MD.** 2014. Characterization of carbapenem-resistant *Acinetobacter baumannii* in Brazil (2008–2011): countrywide spread of OXA-23-producing clones (CC15 and CC79). Diagn. Microbiol. Infect. Dis. **79:**468–472. http://dx.doi.org/10.1016/j.diagmicrobio.2014.03.006.
19. **Revathi G, Siu LK, Lu P-L, Huang L-Y.** 2013. First report of NDM-1-producing *Acinetobacter baumannii* in East Africa. Int. J. Infect. Dis. **17:**e1255-8. http://dx.doi.org/10.1016/j.ijid.2013.07.016.
20. **Walsh TR, Toleman MA, Poirel L, Nordmann P.** 2005. Metallo-beta-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325. http://dx.doi.org/10.1128/CMR.18.2.306-325.2005.
21. **Dalla-Costa LM, Coelho JM, Souza HAPHM, Castro MES, Stier CJN, Bragagnolo KL, Rea-Neto A, Penteado-Filho SR, Livermore DM, Woodford N.** 2003. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J. Clin. Microbiol. **41:**3403–3406. http://dx.doi.org/10.1128/JCM.41.7.3403-3406.2003.
22. **Schimith Bier KE, Luiz SO, Scheffer MC, Gales AC, Paganini MC, Nascimento AJ do, Carignano E, Dalla Costa LM.** 2010. Temporal evolution of carbapenem-resistant *Acinetobacter baumannii* in Curitiba,

southern Brazil. Am. J. Infect. Control **38:**308–314. http://dx.doi.org/10.1016/j.ajic.2009.09.012.

23. **Cieslinski JM, Arend L, Tuon FF, Silva EP, Ekermann RGS, Dalla-Costa LM, Higgins PG, Seifert H, Pilonetto M.** 2013. Molecular epidemiology characterization of OXA-23 carbapenemase-producing *Acinetobacter baumannii* isolated from 8 Brazilian hospitals using repetitive sequence-based PCR. Diagn. Microbiol. Infect. Dis. **77:**337–340. http://dx.doi.org/10.1016/j.diagmicrobio.2013.07.018.
24. **Martins AF, Kuchenbecker RS, Pilger KO, Pagano M, Barth AL.** 2012. High endemic levels of multidrug-resistant *Acinetobacter baumannii* among hospitals in southern Brazil. Am. J. Infect. Control **40:**108–112. http://dx.doi.org/10.1016/j.ajic.2011.03.010.
25. **Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Göttig S, Hunfeld K-P, Seifert H, Witte W, Higgins PG.** 2011. Molecular characterization of *bla*NDM-1 in an *Acinetobacter baumannii* strain isolated in Germany in 2007. J. Antimicrob. Chemother. **66:**1998–2001. http://dx.doi.org/10.1093/jac/dkr256.
26. **Karthikeyan K, Thirunarayan MA, Krishnan P.** 2010. Coexistence of *bla*OXA-23 with blaNDM-1 and *armA* in clinical isolates of *Acinetobacter baumannii* from India. J. Antimicrob. Chemother. **65:**2253–2254. http://dx.doi.org/10.1093/jac/dkq273.
27. **Bonnin R, Poirel L, Nordmann P.** 2014. New Delhi metallo-β-lactamase-producing *Acinetobacter baumannii*: a novel paradigm for spreading antibiotic resistance genes. Future Microbiol. **9:**33–41. http://dx.doi.org/10.2217/fmb.13.69.
28. **Waterman PE, McGann P, Snesrud E, Clifford RJ, Kwak YI, Munoz-Urbizo IP, Tabora-Castellanos J, Milillo M, Preston L, Aviles R, Sutter DE, Lesho EP.** 2013. Bacterial peritonitis due to *Acinetobacter baumannii* sequence type 25 with plasmid-borne New Delhi metallo-β-lactamase in Honduras. Antimicrob. Agents Chemother. **57:**4584–4586. http://dx.doi.org/10.1128/AAC.00275-13.
29. **Pasteran F, Mora MM, Albornoz E, Faccone D, Franco R, Ortellado J, Melgarejo N, Gomez S, Riquelme I, Matheu J, Ramon-Pardo P, Corso A.** 2014. Emergence of genetically unrelated NDM-1-producing *Acinetobacter pittii* strains in Paraguay. J. Antimicrob. Chemother. **69:**2575–2578. http://dx.doi.org/10.1093/jac/dku139.