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Case Report

Earliest identification of New Delhi metallo-β-lactamase 1 (NDM-1) in *Acinetobacter pittii* in Brazil

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Abstract

We report the occurrence in Brazil of the bla_{NDM-1} gene in *Acinetobacter pittii*, prior to the previously described first reports regarding the species *Providencia rettgeri* and *Enterobacter hormaechei*. Clinical isolates were investigated by polymerase chain reaction followed by bidirectional sequencing, and species was confirmed by 16S rDNA sequencing and matrix-assisted laser desorption-ionization time-of-flight spectrometry. *A. pittii* carrying bla_{NDM-1} was confirmed in a patient with no national or international travel history, or transfer from another hospital. The findings warn of the possibility of silent spread of bla_{NDM-1} to the community.

Keywords: NDM-1. Multidrug resistance. *Acinetobacter pittii.*

INTRODUCTION

During the last decade, carbapenem-resistant isolates have become a serious threat to public health worldwide. Since the first identification of the bla_{NDM-1} gene in *Klebsiella pneumoniae* in Sweden in 2009[1] other bacterial species, including NDM-1 producing *Acinetobacter* spp., have been isolated in several parts of the world[2]. The first reports of NDM-1 in Brazil were in 2013 and 2014, in *Enterobacteriaceae* and *Acinetobacter baumannii*, respectively[3-5]. Here, we report the occurrence in 2012 of a clinical isolate of *Acinetobacter pittii* carrying bla_{NDM-1} in a hospitalized patient with no national and international travel history.

CASE REPORT

An 86-year-old woman was admitted for the first time to the emergency room at the Hospital Dona Helena (HDH), Joinville, in southern Brazil in 2005 with a suspected stroke. Imaging tests revealed an ischemic stroke, with severe motor sequelae, speech disorder, and difficulty swallowing.

In September 2012, she was attended in the emergency room at HDH with a decline in her general state of health. Broncho aspiration and hypoxia were confirmed. She was referred to the intensive care unit (ICU) and intubated due to worsening respiratory function and decreased oxygen saturation. After evaluation of the clinical signs and presence of secretion in the orotracheal tube, she was diagnosed with pneumonia and received empirical treatment with 12.5 mL of ampicillin/sulbactam (1 g/500 mg), every 8 hours, while awaiting blood and urine culture results. The urine was positive, with growth of *Proteus mirabilis* above 10^5 colony forming units (CFU)/mL. The isolate was sensitive to ampicillin/sulbactam, cefazolin, ceftriaxone, cefepime, ciprofloxacin, ertapenem, imipenem, and norfloxacin. Treatment with ampicillin/sulbactam was maintained, but the patient's condition worsened after 2 days with development of tachycardia and hypotension. She was infused with 10 mL/hour of dobutamine (12.5 mg/mL) and her condition stabilized. There was rapid weight loss (10 kg) and worsening of swallowing during this period.

On the fifth day of ICU admission there was no improvement of orotracheal secretion. Bronchoalveolar material was sent for

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culture. Multidrug-resistant *Pseudomonas aeruginosa* (4.0×10^4 CFU/mL) and *K. pneumoniae* (8.0×10^4 CFU/mL) resistant only to ampicillin were detected. Aspiration pneumonia was diagnosed, and mechanical ventilation was necessary.

Following treatment, on day 19 of hospitalization, she underwent gastrostomy to reduce the risk of aspiration pneumonia and to improve food support. On day 26, mechanical ventilation was withdrawn and she was transferred to the inpatient ward with tracheostomy. However, on day 42, the clinical condition worsened, evolving to systemic inflammatory response syndrome. She returned to the ICU and treated with piperacillin/tazobactam 4 g/500 mg by prolonged (30-minute) intravenous infusion every 8 hours. She remained febrile with production of orotracheal secretion, and blood and bronchoalveolar lavage cultures were requested. Both cultures were positive for carbapenem-resistant bacteria of the *Acinetobacter calcoaceticus - A. baumannii* complex. The clinical option was to maintain the treatment. On day 50 severe diarrhea developed and she was medicated empirically with metronidazole (400 mg every 8 hours) while awaiting fecal culture results. After a positive result for *Clostridium difficile*, the piperacillin/tazobactam and metronidazole therapy was maintained for 7 days. Clinical improvement occurred and she was discharged.

Investigation of the $bla_{\text{NDM-1}}$ gene was performed by polymerase chain reaction (PCR) using the NDM-F (5'-GGTGCATGCCCGGTGAAATC-3') and NDM-R (5'-ATGCTGGCCTTGGGGAACG-3') primers and previously described thermocycling conditions[1]. A 661 bp segment of the gene was confirmed after agarose gel electrophoresis. The amplicons were bidirectional sequenced using the Big Dye® Terminator v3.1 kit (Thermo Fisher Scientific, Waltham, MA, USA) on the ABI-Prism 3500 Genetic Analyzer platform (Thermo Fisher Scientific) and compared to reference nucleotide sequences of the variants of bla_{NDM} gene available on GenBank (www.ncbi.nlm.nih.gov/genbank/) using the ClustalW software contained in the BioEdit program package version 7.2.6.1.

A. baumannii (5379RM) and *K. pneumoniae* (12491RM) strains carrying the $bla_{\text{NDM-1}}$ gene, provided by the Central Laboratory of the State of Paraná, were employed as positive controls.

The $bla_{\text{NDM-1}}$ gene was confirmed in *A. calcoaceticus - A. baumannii* isolated from blood and bronchoalveolar lavage samples of the same patient and collected on the same day of hospitalization as described above. The isolate harbored the IS*Aba*1 insertion sequence and was negative for all bla_{OXA} genes analyzed, according to multiplex PCR[6].

To speciate the $bla_{\text{NDM-1}}$ positive isolate, approximately 500 bp segments of the 16S rRNA encoding gene were obtained via PCR and amplicons were purified with ExoSAP-IT® (USB Affymetrix). Sequencing was performed using the Microseq 500 16S rDNA kit and BigDye® XTerminator purification kit (Thermo Fisher Scientific) on the ABI 3100 analyzer (Thermo Fisher Scientific). The obtained sequences were compared with the bacterial sequences deposited using the Le Bibi® and Sepsis Blast® systems, which confirmed *A. pittii*. The isolate was also identified by matrix-assisted laser desorption-ionization time-of-flight spectrometry (Vitek-MS®, BioMerieux). The spectrograms were interpreted as previously described[7] using the Vitek-MS® RUO mode of the SARAMIS ™ spectral archive and microbial identification system version 4.12 and enriched by exactly the same SuperSpectrum created based on the selection of 40 specific masses and validated by the authors. This procedure correctly identified species belonging to the *A. calcoaceticus - A. baumannii* complex.

Disk diffusion antibiotic susceptibility tests and automated testing (Vitek 2, BioMerieux®) revealed that *A. pittii* was resistant to meropenem, imipenem, ampicillin/sulbactam, piperacillin/tazobactam, and ceftazidime, and sensitive to polymyxin B, ciprofloxacin, amikacin, gentamicin, doxycycline, sulfamethoxazole/trimethoprim, and tobramycin, according Clinical and Laboratory Standards Institute guidelines[8].

The study received ethical and methodological approval from the Research Ethics Committee of the Universidade da Região de Joinville – Univille (Protocol Nr. 788.455).

DISCUSSION

The $bla_{\text{NDM-1}}$-positive *A. pittii* isolate from a hospitalized patient with no national or international travel history confirmed the occurrence of NDM-1 in Brazil since 2012. In our country, the first isolate harboring the $bla_{\text{NDM-1}}$ gene was reported in 2013, in a diabetic patient hospitalized in Porto Alegre, Rio Grande do Sul, in tissue from a severely injured (and subsequently amputated) foot. *P. rettgeri* resistant to carbapenems was confirmed, with PCR identification of the gene. There were no previous trips to other countries[3]. In the same year, $bla_{\text{NDM-1}}$ in isolates of *Enterobacteriaceae* with reduced susceptibility to carbapenems was reported from 17 hospitals in the same state[4]. Isolates were obtained from cultures of clinical and surveillance materials (rectal swabs and hospital environments). The $bla_{\text{NDM-1}}$ gene was found in six isolates belonging to *Enterobacter cloacae* complex and in two *Morganella morganii* isolates. Most strains were derived from surveillance cultures.

In addition to *Enterobacteriaceae*, NDM-1 has been detected in non-fermenting gram-negative bacilli globally, including China, Egypt, Germany, and Israel[9]. In Brazil, the first *A. baumannii* strain carrying the $bla_{\text{NDM-1}}$ gene was reported in 2014 from the urine of a 71-year-old male with obstructive respiratory disease admitted to the ICU of a hospital in Londrina, Paraná[5]. The $bla_{\text{OXA-51-like}}$ gene was confirmed, but $bla_{\text{OXA23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $\text{bla}_{\text{OXA-143}}$ genes were absent in this isolate. So far, the only previously identified *A. pittii* (ST 119) carrying *bla*NDM-1 gene in Brazilian territory was reported in a colonizing isolate from a 66-year-old man with bladder carcinoma admitted to a tertiary hospital in Porto Alegre, Rio Grande do Sul[10].

It is noteworthy that the $bla_{\text{NDM-1}}$ positive *A. pittii* isolate reported here was initially identified as belonging to the *A. calcoaceticus - A. baumannii* complex, without definition of the species. Most of the phenotypic characteristics among *A. baumannii, A. calcoaceticus, A. pittii, A. dijkshoorniae*, and *A. nosocomialis* are very similar. Thus, identification at the species level inside the complex by conventional methods is

difficult. So, it is reasonable that other *A. pittii* isolates carrying bla_{NDM-1} gene may be spreading in Brazilian hospitals as the majority of clinical laboratories do not determine the species that make up the *A. calcoaceticus - A. baumannii* complex employing molecular techniques[11].

In conclusion, confirmation of the bla_{NDM-1} gene in *A. pittii* isolated from a hospitalized patient in 2012 demonstrates that this carbapenemase was present in Brazilian territory before the previously published date. This finding warns of the possibility of spread to the community, environment, and animals. It also highlights the need for a better understanding of clonal relationships to control dissemination in the hospital environment. The detection of *A. pittii* carrying the bla_{NDM-1} gene occurred in a patient with no national or international travel history or a transfer from another hospital center. This prompts concern about the lack of definition of its possible reservoirs.

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Conflict of interest

The authors declare that there is no conflict of interest.

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