NDM-1 carbapenemase in *Acinetobacter baumannii* sequence type 32 in Ecuador

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Abstract

Objectives: To describe a clinical case of *Acinetobacter baumannii* sequence type (ST) 32 harbouring a New Delhi metallo-β-lactamase (NDM) in Ecuador.

Methods: We used multilocus sequence typing (MLST) to confirm the bacterial species and the sequence type of an *A. baumannii* isolate. We used synergy with the imipenem–EDTA disc method and the carbapenem inactivation method (CIM) to determine carbapenemase production; the presence of a carbapenemase gene was confirmed by PCR amplification and amplicon sequencing.

Results: Molecular characterization revealed the presence of *A. baumannii* ST32 harbouring the bla_{NDM-1} gene in Ecuador. The bla_{NDM-1} gene was isolated through PCR and amplified from a purified plasmid.

Conclusions: To the best of our knowledge, this is the first report of *A. baumannii* ST32 harbouring the $\boldsymbol{bla_{NDM-1}}$ gene.

Keywords: *Acinetobacter baumannii*, antibiotic resistance, blaNDM-1, MSLT, sequence type 32
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The New Delhi metallo-β-lactamase (NDM) is a transferable molecular class B carbapenemase (zinc metallo-β-lactamase) reported in Sweden in 2008 in a *Klebsiella pneumoniae* isolate from an Indian patient [1]. To date, NDM carbapenemases have been reported in most regions around the world owing to the rapid dissemination of the gene between members of the Enterobacteriaceae and *Acinetobacter* spp. in human and environmental isolates [2–4].

In Latin America and the Caribbean, NDM carbapenemase has been detected in many isolates of *A. baumannii* (Brazil, Colombia, and Honduras), *A. bereziniae* (Argentina, Brazil) and *A. pittii* (Paraguay) [5]. We recently reported the presence of the bla_{NDM-1} gene in *A. baumannii* in Ecuador without further information on the isolate [6]. Here, we report the laboratory and clinical context of a bla_{NDM-1} gene from *A. baumannii* sequence type (ST) 32 infecting an English patient traveling from Amsterdam to Ecuador.

After a 4-hour flight, a 59-year-old man presented psychomotor agitation with a deterioration in his state of consciousness that progressed with time. The patient had a history of atrial fibrillation which was under treatment. When he arrived in Ecuador, he was immediately assisted and transferred to the emergency ward at the Hospital de los Valles in Quito. He presented a Glasgow Coma Score (GCS) of 5/15 and developed an ischaemic stroke requiring a tracheostomy and gastrostomy at the intensive care unit (ICU). On day 2 of hospitalization he developed an acute tracheobronchitis associated with mechanical ventilation. A tracheal aspirate and blood samples were sent to the laboratory and an empirical antibiotic therapy of ampicillin–sulbactam (3 g intravenously every 6 h) was initiated. A culture of tracheal aspirate revealed *Serratia marcescens* and the antimicrobial therapy was changed to piperacillin–tazobactam (4.5 g intravenously every 6 h). Twelve days after hospitalization of the patient, *Pseudomonas aeruginosa* was isolated from a blood sample and the same antibiotic regimen was continued owing to *in vitro* susceptibility of the *P. aeruginosa* strain. On day 15 the patient developed fever (37.9°C), and a peripheral central venous catheter, a catheter tip, and urine samples were sent

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TABLE 1. Clinical milestones during patient treatment in Ecuador. Antibiotic treatment was initiated according to the Hospital de los Valles scheme of susceptibility. *Acinetobacter baumannii* infection started on day 15 after hospitalization (bold)

Historical record	Date	Clinical manifestations	Location	Antibiotic treatment	Comments
Day 1	15 May	Psychomotor agitation with deterioration of the state of consciousness	Airport, Emergency room, ICU		Diagnosis of ischaemic stroke requiring mechanical ventilation, tracheostomy and gastrostomy
Day 3	17 May	Suspected diagnosis of ventilator-associated pneumonia (VAP). Empirical antibiotic treatment initiated	ICU	Ampicillin/sulbactam (3 g i.v. every 6 h)	Tracheal aspirate and blood samples sent to laboratory
Day 7	21 May	*Serratia marcescens* isolated from tracheal aspirate culture	ICU	Piperacillin/tazobactam (4.5 g i.v. every 6 h)	
Day 12	26 May	*Pseudomonas aeruginosa* isolated from blood culture. Same antibiotic regimen	ICU	Piperacillin/tazobactam (4.5 g i.v. every 6 h)	
Day 15	**29 May**	The patient developed fever (37.9°C), white blood cells = 11,400 cells/μL, procalcitonin = 0.48 ng/mL (ascending)	ICU	Meropenem (1 g i.v. every 8 h)	Peripheral, central venous catheter, catheter tip, blood and urine samples sent to laboratory
Day 18	1 June	*A. baumannii* isolated from blood samples	ICU	Meropenem (1 g i.v. every 8 h) Colistin (100 mg i.v. every 8 h)	Absence of urinary or respiratory reinfections
Day 27	10 June	Patient's clinical condition improved		Meropenem (1 g i.v. every 8 h) Colistin (100 mg i.v. every 8 h)	Patient transferred to London

to the laboratory because of a suspicion of septicaemia (Table 1). Microbiological cultures revealed carbapenem-resistant *A. baumannii* (Vitek 2; bioMérieux). At this point, the piperacillin–tazobactam combination was changed to meropenem (1 g intravenously every 8 h) followed by colistin (100 mg intravenously every 8 h) and strict infection control measures (emphasizing contact precautions) were implemented.

The isolate was submitted to the National Reference Laboratory of Antimicrobial Resistance (Instituto Nacional de Investigación en Salud Pública 'Leopoldo Izquieta Perez', Quito-Ecuador) for molecular analysis of the resistance genes. The *A. baumannii* strain was identified by polymerase chain reaction (PCR) of $bla_{\text{OXA-51}}$-like and *gyrB* genes, which confirmed the species of bacterium. Antimicrobial susceptibility testing was conducted using the disk diffusion method and automated testing (Vitek2 AST-N272). Results revealed resistance to ceftazidime (MIC 64 μg/mL), cefepime (MIC 64 μg/mL), imipenem (MIC 8 μg/mL), meropenem (MIC 16 μg/mL), piperacillin–tazobactam (MIC 128/4 μg/mL), intermediate resistance to ampicillin–sulbactam (MIC 16/8 μg/mL), and susceptibility to gentamicin (MIC $\leq$1 μg/mL), ciprofloxacin (MIC 1 μg/mL), tigecycline (MIC 2 μg/mL), and colistin (MIC 0.5 μg/mL) according to *Acinetobacter* spp. breakpoints [7].

Phenotypic screening for carbapenemase production using EDTA [8] showed a synergy between imipenem and meropenem discs; moreover, the carbapenem inactivation method (CIM) was positive. Screening for carbapenemase genes (bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{VIM}, bla_{SIM}, bla_{PER}, bla_{VEB}, bla_{GIM}, bla_{GES}) by PCR revealed the presence of bla_{NDM}; the 947-base-pair amplicon was sequenced and confirmed as $bla_{\text{NDM-1}}$ allele (Genebank accession no: MF038874). The exploration of class D β-lactamases ($bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-24}}$-like, $bla_{\text{OXA-58}}$-like and $bla_{\text{OXA-143}}$) using a protocol based on Woodford et al. (2006) [9,10] yielded negative results. Conjugation assays with *Escherichia coli* J53 as the recipient bacterium and transformation experiments with *E. coli* DH5α strain failed; however, we amplified $bla_{\text{NDM-1}}$ from purified plasmid DNA (extracted with PureYield™ Plasmid Miniprep System and separated by electrophoresis), suggesting that the $bla_{\text{NDM-1}}$ gene is located in a plasmid. A multilocus sequence typing (MLST) analysis of the *A. baumannii* strain based on the Pasteur Institute scheme (http://www.pasteur.fr) was performed and showed the profile *cpn60* (1), *fusA* (1), *gltA* (2), *pyrG* (2), *recA* (3), *rplB* (4), *rpoB* (4), indicating that the isolate belonged to the sequence type 32 (ST32).

On day 27 the patient was discharged from the ICU due to an improvement in his clinical condition. His treatment continued with meropenem (1 g intravenously every 8 h) followed by colistin (100 mg intravenously every 8 h) and was immediately transferred to London with contact precautions and the appropriate medical care.

To the best of our knowledge, this is the first description of *A. baumannii* ST32 harbouring the $bla_{\text{NDM-1}}$ gene in Ecuador and possibly in the world; interestingly, previous reports highlight the importance of carbapenem-resistant *A. baumanni* clonal complex 32 (ST32, ST28, ST138) as a new emerging international clone IV with epidemic potential [11]. No similar isolates have been detected in this healthcare centre; however, the patient had recognized risk factors associated with this type of infection (mechanical ventilation, previous antibiotic exposure, ICU hospitalization), which may indicate that this was a local infection in a foreign patient (Table 1), adding more evidence for the endemicity of NDM-1 in Ecuadorian hospitals [6,12]. We were unable to demonstrate the transferability of $bla_{\text{NDM-1}}$ in the present case. Similarly, pNDM-BJ01-like plasmids

harbouring bla_{NDM} genes were recently reported in *Acinetobacter* spp. [13]; in some of them the conjugation-related genes are truncated or deleted, which affects their mobilization [14]. Detection of metallo-β-lactamases should continue to be prioritized since the hazards of multiple resistance genes spreading in multiple bacterial species is an undetected reality that it is challenging to anticipate and control.

Ethical approval

The development and publication of the manuscript was approved by Dr Harry F. Dorn, medical Director of Hospital de los Valles (Quito, Ecuador), who declared that the manuscript is within the hospital's ethical policies; individual patient compromising data is absent.

Transparency declaration

The authors declare that they have no conflict of interests. This study was funded by the Instituto Nacional de Investigación en Salud Pública 'Dr Leopoldo Izquieta Pérez', Quito, Ecuador and Pontificia Universidad Católica del Ecuador grant M13455.

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