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Short Communication

First case of New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* from Ecuador: An update for South America

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ABSTRACT

Objectives: To describe a clinical case of *Klebsiella pneumoniae* harboring a New Delhi metallo-β-lactamase (NDM) plasmid in Ecuador and to present a map of reports of NDM isolates in South America.
Methods: The modified Hodge test, carbapenem inactivation method, imipenem–EDTA disk method (synergy), and Rapidec Carba NP test were used to identify antibiotic resistance mechanisms. The presence of resistance genes was explored with a conjugation assay, and molecular confirmation of NDM was performed by PCR and DNA sequencing. Plasmid characterization was conducted by PCR-based replicon typing. A literature review was performed in Google Scholar and PubMed to identify reports from South America.
Results: An HIV-infected patient, who had never traveled abroad, developed a bloodstream infection caused by *K. pneumoniae* ST147 harboring the NDM-1 resistance gene in a plasmid from the IncA/C group. Local circulation of NDM has also been described in other South American countries, in particular in Colombia and Brazil, although published scientific records were not found for other countries.
Conclusions: This report presents the first evidence of autochthonous circulation of the NDM-1 resistance gene harbored by an IncA/C plasmid isolated from a *K. pneumoniae* ST147 in Ecuador. Efforts should be implemented to monitor and characterize the spatial and temporal distribution of NDM in Ecuador and other countries of South America.

Introduction

Antibiotic resistance is a worldwide concern due to the global distribution of multiple plasmids spreading among different bacterial families (Logan and Weinstein, 2017). Infections with carbapenemase-producing bacteria increase mortality and morbidity rates among those infected and therefore place a high burden on the health sector (Villegas et al., 2016). New Delhi metallo-β-lactamase (NDM) is one of the most recent class B carbapenemases to be identified and confers resistance to all β-lactam antibiotics except aztreonam (Patel and Bonomo, 2013). Since its first detection in 2008, NDM has been found in several localities across the five continents (Logan and Weinstein, 2017).

In South America, NDM was first reported in Colombia (Pérez et al., 2013). A map of the scientific literature reporting NDM in South America up until April 30, 2017 was produced (Figure 1; **Supplementary Material**, Table S1). In Ecuador, the NDM gene was recently found in the bacteria *Providencia rettgeri* (Zurita et al., 2015) and *Acinetobacter baumannii* (GenBank accession number **MF038874**) without confirmation of a plasmid. The presence of plasmid-borne NDM in a *Klebsiella pneumoniae* ST147 isolate from a patient hospitalized in Ecuador is reported herein.

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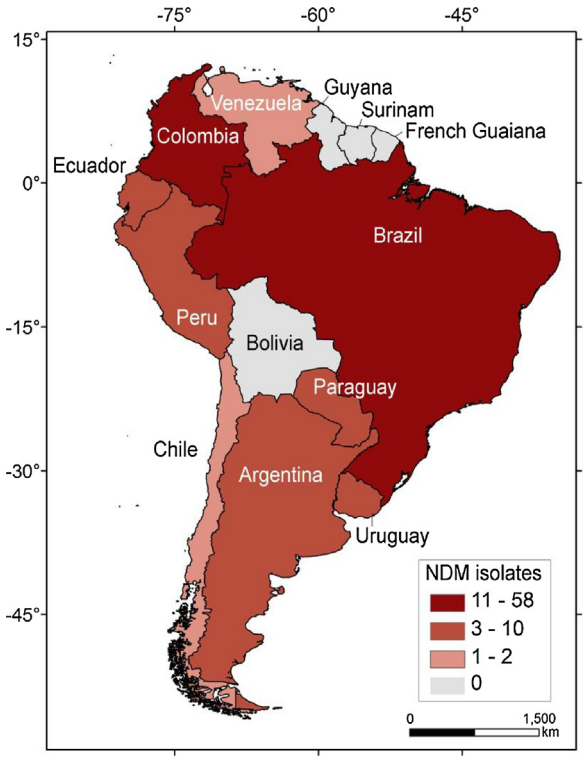

Figure 1. Current distribution of New Delhi metallo-β-lactamase (NDM) reports in South America. Reports were collected through a literature review in the Google Scholar and PubMed databases, resulting in 33 published reports (**Supplementary Material**, Table S1). No records were found for four South American countries (grey).

Case report

A 30-year-old male patient, with no history of international travel, was diagnosed with an HIV infection in 2012. Adherence to his antiretroviral treatment was continuously suboptimal. In November 2016, the patient was hospitalized in a public hospital in the province of Esmeraldas northern Ecuador due to headache, blindness, and hearing loss. He spent 8 days there before being transferred to Quito, the capital city of Ecuador in the center of the country. There, cerebral imaging studies revealed an absence of space-occupying lesions. *Cryptococcus neoformans* was isolated from the patient's cerebrospinal fluid and a treatment regimen based on amphotericin B deoxycholate + fluconazole was initiated. He also received lopinavir/ritonavir and lamivudine/abacavir for his HIV infection and daily doses of trimethoprim–sulfamethoxazole considering his immunosuppressed status.

Five days after the patient was transferred to Quito, a central venous catheter (CVC) was placed due to difficulty gaining a peripheral venous access. Forty-one days later, the patient developed chills, fever, and showed a decrease in his mental faculties. Two blood cultures and a culture of the CVC after extraction were sent to the microbiology laboratory, where a carbapenemase-resistant *K. pneumoniae* was identified using the VITEK 2 Compact card AST-N272 (bioMérieux, Marcy l'Etoile, France). This *K. pneumoniae* showed resistance to carbapenems (e.g., imipenem and meropenem ≥16 μl/ml, ertapenem 2 μg/ml), cephalosporins (e.g., ceftriaxone ≥64 μg/ml), and quinolones (e.g., ciprofloxacin ≥4 μg/ml), and was sensitive to aztreonam (24 mm detected by Kirby–Bauer method according to the Clinical and Laboratory Standards Institute CLSI (2017)).

The following techniques were used to identify carbapenemase activity: the modified Hodge test and carbapenem inactivation method in accordance with CLSI (2017), the synergy by imipenem–ethylenediaminetetraacetic acid (EDTA) disk method (Yong et al., 2002), and the Rapidec Carba NP test following the manufacturer's instructions (bioMérieux) (Table 1). The *K. pneumoniae* was identified as the variant sequence type (ST) 147 via multilocus sequence typing (MLST), according to the Pasteur scheme (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). The carbapenemase genes bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{NDM} were explored using primers described elsewhere (Poirel et al., 2011), and only the NDM-1 carbapenemase gene was identified (GenBank accession number **MF038875**). A conjugation assay was performed using the broth-mating technique, with an *Escherichia coli* J53 as the recipient bacterium. The Sensititre system with ARGNF plates (Thermo Fisher Scientific, Massachusetts, USA) was used to determine antibiotic susceptibility at this point (Table 1). Transconjugant strains were cultivated in MacConkey agar supplemented with sodium azide (100 mg/l) and meropenem (0.5 mg/l) (Table 1). The presence of the bla_{NDM} gene in transconjugant strains was confirmed using PCR. Furthermore, PCR-based replicon typing (PBRT) was used to characterize the plasmid from the recipient *E. coli* J53 (Carattoli et al., 2005), which is part of the incompatibility group A/C (IncA/C).

Once the *K. pneumoniae* had been identified, the patient was started on triple antibiotic therapy with oral fosfomycin (1 g every 8 h) and intravenous meropenem (1 g every 8 h) and colistin methanesulfonate (150 mg every 8 h). At the completion of antibiotic treatment (i.e., 21 days), blood cultures and rectal swabs were negative for *K. pneumoniae*. Seventy-seven days after his hospitalization, the patient was transferred to another hospital to continue treatment for cryptococcal meningitis and HIV.

Table 1
Characteristics of the isolated *Klebsiella pneumoniae* ST147 and its transconjugant: MICs (μg/ml), carbapenemase activity test results, and resistance genes.[a]

Antimicrobial agent	*K. pneumoniae* ST147	Transconjugant	*E. coli* J53
MIC (μg/ml)			
Imipenem	≥8	2	≤0.5
Meropenem	4	2	≤1
Ertapenem	≥2	≥2	≤1
Aztreonam	≥16	≤8	≤8
Ceftazidime	≥32	≤2	≤2
Cefepime	8	≤2	≤2
Piperacillin–tazobactam	>64/4	32/4	≤8/4
Amikacin	≥32	8	≤8
Gentamicin	≥8	≤4	≤4
Ciprofloxacin	≥2	≥2	≤0.06
Tigecycline	1	≤0.5	≤0.5
Colistin	≤1	≤1	≤1
Carbapenemase test			
Modified Hodge test	Positive	Positive	Negative
CIM	Positive	Positive	Negative
Boronic acid synergy	Negative	ND	ND
EDTA synergy	Positive	Positive	Negative
Rapidec Carba NP	Positive	Positive	Negative
Resistance genes			
bla_{KPC}	Negative	Negative	ND
bla_{NDM-1}	Positive	Positive	ND
bla_{IMP}	Negative	Negative	ND
bla_{VIM}	Negative	Negative	ND
bla_{OXA-48}	Negative	Negative	ND
bla_{CTX-M}	Positive	Negative	ND
bla_{SHV}	Positive	Negative	ND

MIC, minimum inhibitory concentration; CIM, carbapenem inactivation method; ND, not determined; EDTA, ethylenediaminetetraacetic acid; CLSI, Clinical and Laboratory Standards Institute.

[a] Susceptibility tests were performed using Sensititre ARGNF plates, and interpretation was based on the CLSI breakpoints of 2017. Breakpoints for tigecycline and colistin were those of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/).

Due to the novelty of this plasmid-borne resistance pattern for Ecuador, strict control measures were implemented, including contact precautions, exclusive nursing personnel and vital sign measurement equipment, the restriction of incoming patients in the area, and the provision of rigorous instructions on hand hygiene and isolation methods to people involved in the patient's healthcare. The patient's spouse and other patients sharing nursing personnel on the same floor (three in total) were examined with rectal swabs to identify colonization by Enterobacteriaceae, and all results were negative.

Discussion

This report provides the first evidence of the autochthonous circulation of NDM-1 harbored by a plasmid of the IncA/C group isolated from a *K. pneumoniae* ST147 causing a bloodstream infection in an HIV-positive patient in Ecuador. This case should be considered as further evidence of the autochthonous circulation of the NDM-1 plasmid in Gram-negative bacilli in Ecuador, since the patient had never travelled abroad.

Public hospitals in Ecuador should have the ability to detect metallo-β-lactamase enzymes in order to prevent outbreaks. Furthermore, affordable techniques should be implemented, such as the carbapenem inactivation method, which can consistently detect both serine- and metallo-β-lactamase enzymes (Logan and Weinstein, 2017). NDM-1 typically confers a broad spectrum of antibiotic resistance compared to other metallo-β-lactamases (Patel and Bonomo, 2013). Its presence in a *K. pneumoniae* ST147 epidemic strain should be of concern considering the widespread distribution of this bacterium across Ecuador harboring other resistance plasmids (Zurita et al., 2013).

The review of the literature showed that local circulation has also been described in other South American countries, in particular in Colombia and Brazil, where several species of Gram-negative bacilli have been found to harbor the NDM gene (**Supplementary Material**, Table S1). The lack of reports from other South American countries should be considered with caution, due to the variability of surveillance efforts among Latin American countries (Escobar et al., 2016), and the limited efforts made by some of them to publish in international indexed journals (e.g., Ecuador vs. Brazil, see **Supplementary Material**, Table S1). Efforts to proactively identify the distribution of multidrug-resistant Enterobacteriaceae carrying NDM-1 should be made in Ecuador in order to characterize its prevalence, anticipate future hospital outbreaks, inform risk for international travelers, and develop a stronger network of collaboration for the public health response to antibiotic-resistant bacteria.

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Ethical approval

Santiago Echeverría MD, Assistant Director of Hospital General Enrique Garcés (Quito, Ecuador), approved the development and publication of the manuscript, which is within the ethical policies of the institution, and has declared the absence of compromising data. Further, individual patient consent was obtained for the publication of this article.

Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2017.10.012.

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