Title

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

**Running title:** NDM in South America

Authors

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Highlights
- New Delhi metallo-β-lactamase (NDM) resistance plasmid has autochthonous circulation in Ecuador.
- *Klebsiella pneumoniae* ST147 harboring NDM-1 gene in an IncA/C plasmid is described for the first time in Quito, Ecuador.
- NDM circulation in South America has been addressed mainly by Brazil and Colombia.

Abstract

**Objectives:** To describe a clinical case of *Klebsiella pneumoniae* harboring New Delhi metallo-β-lactamase (NDM) plasmid in Ecuador and to present a map of reports of NDM isolates in South America.

**Methods:** We used the Modified Hodge Test, Carbapenem Inactivation Method, the synergy with imipenem-EDTA disk method, and Rapidec Carba NP for identification of antibiotic resistance mechanisms. The presence of resistant genes was explored with a conjugation assay, and molecular confirmation of NDM with PCR and DNA sequencing. Plasmid characterization was performed with PBRT. Literature review for reports in South America was performed in Google Scholar and PubMed.

**Results:** An HIV patient that has never traveled abroad developed a blood stream infection caused by *K. pneumoniae* ST147 harboring the NDM-1 resistant gene in a plasmid from the IncA/C group. Local circulation of NDM has also been described in other South American countries especially in Colombia and Brazil although we did not find scientific published records from other countries.

**Conclusions:** We present the first evidence of autochthonous circulation of the NDM-1 resistant 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.
Keywords. NDM, South America, *Klebsiella pneumoniae*, antibiotic resistance, plasmid

Main Text

Antibiotic resistance is a worldwide concern due to the global distribution of multiple plasmids spreading among different bacterial families [1]. Infections with carbapenemase producing bacteria increase mortality and morbidity rates among those infected and therefore impose a high burden over the health sector [2]. New Delhi metallo-β-lactamase (NDM) is one of the most recent class B carbapenemases detected, conferring resistance to all β-lactams antibiotics except aztreonam [3]. Since its first detection in 2008, NDM has been found in several localities in the five continents [1]. In South America, NDM was reported for the first time in Colombia [4]. We developed a map of the scientific literature reporting NDM in South America by April 30th, 2017 (Figure 1 and supplementary table). In Ecuador, NDM gene was recently found in the bacterium *Providencia rettgeri* [5] and *Acinetobacter baumannii* (GenBank accession no: MF038874) without confirmation of a plasmid. We report the presence of plasmid-borne NDM in a *Klebsiella pneumoniae* ST147 in a hospitalized patient in Ecuador.

A 30-year old male patient without history of international travel was diagnosed with HIV infection in 2012 and never reached appropriate adherence to his antiretroviral treatment. In November 2016, the patient was hospitalized for cephalea, blindness, and hearing loss in a public hospital in the province of Esmeraldas northern Ecuador. He spent 8 days there before being transferred to Quito, Ecuador’s capital, in the center of the country. There, cerebral image studies yielded absence of occupative lesions. *Cryptococcus neoformans* was isolated from patient’s cerebrospinal fluid and a treatment based on amphotericin B deoxycholate + fluconazole was initiated. He
received lopinavir/ritonavir and lamivudine/abacavir for HIV and daily doses of trimethoprim sulfamethoxazol considering his immunosuppressive status. Five days after the patient was transferred to Quito, a central venous catheter (CVC) was set due to difficult 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 identifying a carbapenemase resistant *K. pneumoniae* using VITEK®2 Compact (bioMérieux, Marcy l'Étoile, France) card AST-N272, showing 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 being sensitive to aztreonam (i.e., 24 mm detected by Kirby Bauer method according to CLSI 2017 [6]).

We used the following techniques to identify carbapenemase activity: the Modified Hodge Test and Carbapenem Inactivation Method following CLSI 2017 [6], the synergy with imipenem-EDTA disk method [7], and the Rapidec Carba NP test following the manufacturer instructions (bioMérieux) (Table 1). We identified *K. pneumoniae* as the variant sequence type (ST) 147 via Multilocus Sequence Typing (MLST), according to Pasteur’s scheme (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). We searched the carbapenemase genes *bla*KPC, *bla*IMP, *bla*VIM, and *bla*NDM using primers described elsewhere [8], identifying only the NDM-1 carbapenemase gen (GenBank accession no. MF038875). We performed a conjugation assay using the broth-mating technique with an *Escherichia coli* J53 as the recipient bacterium. We used Sensititre® with ARGNF plates (Thermo Fisher Scientific, Massachusetts, USA) to determine antibiotic susceptibility at this point (Table 1). Transconjugants strains were cultivated in McConkey Agar supplemented with sodium azide (100 mg/L) and meropenem (0.5
mg/L) (Table 1); we confirmed the presence of the \textit{bla}_{\text{NDM}} \text{ gene in transconjugants}
strains using PCR. We further used PCR-based replicon typing (PBRT) to characterize the
plasmid from the recipient \textit{E. coli J53} [9], which is part of the incompatibility group
A/C (IncA/C).

Once \textit{K. pneumoniae} was identified, triple antibiotic therapy for the patient was initiated
with oral fosfomycin (1 g every 8 h.), and intravenous meropenem (1 g every 8 h.) and
colistin methanesulfonate (150 mg every 8 h.). After antibiotic treatment completion
(i.e., 21 days), blood cultures and rectal swabs were negative to \textit{K. pneumoniae}.
Seventy-seven days after his hospitalization the patient was transferred to another
hospital to continue the treatment for cryptococcal meningitis and HIV. Due to the
novelty of this plasmid-borne resistance pattern for Ecuador, the implementation of
control measures was strict and included contact precautions, exclusive nursing personal
and vital sign measure equipment, restriction of incoming patients in the area, and
rigorous instructions about hand hygiene and isolation methods offered to the people
involved in the patient’s health care. The patient’s spouse and other patients sharing
nursing personnel on the same floor (three in total) were examined with rectal swabs to
determine Enterobacteriaceae colonization with negative results.

We present the first evidence of autochthonous circulation of NDM-1 harbored by a
plasmid of the IncA/C group isolated from a \textit{K. pneumoniae} ST147 producing a blood-
stream infection in an HIV patient from Ecuador. This case should be considered as
further evidence of the autochthonous circulation of the NDM-1 plasmid in gram-
negative bacilli of Ecuador since the patient has never travelled abroad. Ecuadorian
public hospitals should have the ability to detect metallo-\(\beta\)-lactamase enzymes in order
to prevent outbreaks. This advice may be followed with the implementation of affordable techniques such as the Carbapenem Inactivation Method, which can consistently detect both serine- and metallo-$\beta$-lactamase enzymes [1]. NDM-1 typically confers a broad spectrum of antibiotic resistance compared to other metallo-$\beta$-lactamases [3]; its presence in K. pneumoniae ST147 epidemic strain should be of concern considering the widespread distribution of this bacterium across Ecuador harboring other resistant plasmids [10]. Our literature review showed that local circulation has also been described in other South American countries especially in Colombia and Brazil where several species of gram-negative bacilli have been found to harbor the NDM gene (supplementary table). The lack of reports in other South American countries should be considered with caution due to the variability of surveillance efforts among Latin American countries [11], and the limited efforts by some of them to publish in international indexed journals (e.g., Ecuador vs. Brazil, see supplementary table). Efforts to proactively identify the distribution of multidrug resistant Enterobacteriaceae carrying NDM-1 should be implemented in Ecuador to characterize its prevalence, anticipate future hospital outbreaks, inform risk to international travelers, and develop a stronger network of collaboration for 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 declare the absence of compromising data. Further, individual patient consent was obtained for the publication of this article.

Conflict of interests
Authors declare that there is no conflict of interest.

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References


Figure 1: Current distribution of NDM reports in South America. Reports were collected according to a literature review in Google Scholar and PubMed databases, resulting in 33 published reports (supplementary table). We were unable to find records for four South American countries (grey).
Table 1: Minimum inhibitory concentration (µg/mL), carbapenemase activity tests, and resistance genes of the isolated *Klebsiella pneumoniae* ST147 and its transconjugant. Susceptibility tests were performed using Sensititre® (Thermo Fisher Scientific) plate ARGNF, and interpretation was based on Clinical Laboratory Standards Institute (CLSI) break points 2017. Breakpoints for tigecycline and colistin were based according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, http://www.eucast.org/). MIC*: minimum inhibitory concentration; CIM†: carbapenem inactivation method; Nd‡: not determined.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th><em>K. pneumoniae</em> ST147</th>
<th>Transconjugant</th>
<th><em>E. coli</em> J53</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC</strong> (µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>≥8</td>
<td>2</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>4</td>
<td>2</td>
<td>≤1</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≥2</td>
<td>≥2</td>
<td>≤1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥16</td>
<td>≤8</td>
<td>≤8</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥32</td>
<td>≤2</td>
<td>≤2</td>
</tr>
<tr>
<td>Cefepime</td>
<td>8</td>
<td>≤2</td>
<td>≤2</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64/4</td>
<td>32/4</td>
<td>≤8/4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥32</td>
<td>8</td>
<td>≤8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥8</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>≥2</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1</td>
<td>≤0.5</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Colistin</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
</tr>
</tbody>
</table>

**Carbapenemase Test**

<p>| Modified Hodge Test           | Positive | Positive | Negative |</p>
<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boronic Acid sinergy</td>
<td>Negative</td>
<td>Nd\textsuperscript{1}</td>
<td>Nd\textsuperscript{1}</td>
</tr>
<tr>
<td>EDTA sinergy</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Rapidec Carba NP</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Resistance Genes**

| Gene          | Positive | Positive | Negative | Nd |
|---------------|----------|----------|----------|
| \( blakPC \)  | Negative | Negative | Nd       |
| \( blan\textsubscript{NDM-1} \) | Positive | Positive | Nd       |
| \( bla\textsubscript{IMP} \)   | Negative | Negative | Nd       |
| \( blavIM \)  | Negative | Negative | Nd       |
| \( blao\textsubscript{OXA-48} \) | Negative | Negative | Nd       |
| \( blactX.M \) | Positive | Negative | Nd       |
| \( blah\textsubscript{SHV} \) | Positive | Negative | Nd       |