**bla**<sub>NDM-1</sub>-positive Klebsiella pneumoniae from Environment, Vietnam

To the Editor: The **bla**<sub>NDM-1</sub> gene, which produces the New Delhi metallo-β-lactamase (NDM-1) enzyme, confers resistance to the carbapenem class of antimicrobial drugs and can be transferred among different types of bacteria. NDM-1 was identified in 2008 in Sweden from a patient from India who had been hospitalized in New Delhi (1). Since that report, **bla**<sub>NDM-1</sub>-positive bacteria have been identified from patients in several countries; most of these patients had a direct link with the Indian subcontinent (2). The spread of **bla**<sub>NDM-1</sub> among bacterial pathogens is of concern not only because of resistance to carbapenems but also because such pathogens typically are resistant to multiple antimicrobial drug classes, which leaves few treatment choices available (3-5).

In 2011, spread of **bla**<sub>NDM-1</sub>-positive bacteria in an environmental setting in New Delhi was reported (6).

The possible appearance of bacteria harboring **bla**<sub>NDM-1</sub> in Vietnam is of concern because cultural and economic links between Vietnam and India are strongly established, including extensive person-to-person exchanges that could enable easy exchange of pathogens. In addition, Vietnam faces a serious problem of antimicrobial drug resistance because drugs are freely available and used in an indiscriminate fashion. Thus, once **bla**<sub>NDM-1</sub>-positive bacteria colonize persons in Vietnam, they would be able to spread easily and pose a serious public health threat.

During September 2011, we collected paired swab samples (1 for PCR, 1 for culture) of seepage water from 20 sites (rivers, lakes, and water pools in streets) within a 10-km radius of central Hanoi, Vietnam. Samples were transported in Transystem (COPAN Italia S.p.A, Brescia, Italy) to preserve bacteria and DNA. The 20 PCR swab specimens were squeezed out into 0.5-mL volumes of sterile water and centrifuged at 3,000 × g for 30 seconds; 1 μL of the resulting suspension was then used as PCR template to detect **bla**<sub>NDM-1</sub> as described (7). Two samples were positive for **bla**<sub>NDM-1</sub>; these 2 samples were collected from the same river (Kim Ngua River) but at sites 3 km apart.

To isolate and identify the phenotype and genotype of **bla**<sub>NDM-1</sub>-positive bacteria, we repeatedly spread the 20 culture swab specimens onto Muller-Hinton agar (Nissui, Tokyo, Japan) containing 100 mg/L vancomycin (Nakalai, Kyoto, Japan) plus 0.5 mg/L meropenem (LKT Laboratories, St. Paul, MN, USA) until single colonies were obtained. Each colony was then subcultured by plating onto MacConkey agar (Nihon Seiyaku, Tokyo, Japan) containing 0.5 mg/L meropenem to ensure culture purity; colonies were identified by using API 20E strips (bioMérieux, Basingstoke, UK). MICs of these isolates for 13 antimicrobial drugs were calculated by using Etest (bioMérieux), and susceptibility data were interpreted by using Clinical and Laboratory Standards Institute guidelines (www.clsi.org).

We harvested several species of bacteria from the 2 seepage samples positive for **bla**<sub>NDM-1</sub>: Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. fluorescens/putida, and P. luteola. These isolates were plated onto media containing 0.5 mg/L meropenem, and bacterial DNA was extracted and used for the template for PCR analysis to detect **bla**<sub>NDM-1</sub> as described (7). **bla**<sub>NDM-1</sub> was detected in 3 K. pneumoniae isolates from each of the 2 positive samples (6 isolates total); this result was confirmed by sequencing. All 6 isolates were highly resistant to all β-lactam antimicrobial drugs, including carbapenems (Table). To
detect another $\beta$-lactamase, multiplex PCRs were carried out as described (8); genetic variants bla$_{TEM}$, bla$_{SHV}$, bla$_{CTX-M}$, bla$_{IMP}$, bla$_{ARM}$, and bla$_{PER}$ were not detected in any of the isolates other than K. pneumoniae. All 6 K. pneumoniae isolates were positive for bla$_{TEM}$ and bla$_{CTX-M}$ variants by PCR; these variants were confirmed as bla$_{TEM}$ and bla$_{CTX-M}$ by sequencing. Aminoglycosides are often used in the management of severe infectious diseases caused by gram-negative pathogens. 16S rRNA methylases were found to confer high levels of resistance to aminoglycosides such as amikacin, tobramycin, and gentamicin. The 6 K. pneumoniae isolates we found were highly resistant to gentamicin (MIC $>1,024$ mg/L) and tobramycin (MIC $256\rightarrow1,024$ mg/L) (Table). Therefore, we screened genetic elements of 16S rRNA methylases (rmtB, rmtC, and armA) by PCR and detected rmtB in all 6 isolates (9). Multilocus sequence typing was applied for these 6 isolates; all were identified as K. pneumoniae sequence type 283 (10), which had not been reported as harboring bla$_{NDM}$. The azide-resistant Escherichia coli strain 153 has been used as recipient for conjugation assay, which had been reported previously (6), but we found no transconjugant strain with bla$_{NDM}$ on MacConkey agar containing 100 mg/L sodium azide and 0.5 mg/L meropenem.

Our results show that bla$_{NDM}$--positive K. pneumoniae sequence type 283 is present in the Kim Nggu River, which flows through the central part of Hanoi at 2 sites. The isolates we obtained were also positive for 2 other $\beta$-lactamases, bla$_{TEM}$ and bla$_{CTX-M}$, were highly resistant to aminoglycosides related to rmtB, and showed mild elevation of MIC against ciprofloxacin up to 1.5 mg/L. Wide-scale surveillance of environmental and clinical samples in Vietnam and establishment of a strategy to prevent further spread of bla$_{NDM}$ are urgently needed.

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**References**


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Table. Resistance to 13 antimicrobial drugs of bla$_{NDM}$--positive Klebsiella pneumoniae isolates from the Kim Nguu River, Hanoi, Vietnam

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>MIC, mg/L</th>
<th>Site X</th>
<th>MIC, mg/L</th>
<th>Site Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin/tazobactam</td>
<td>$64\rightarrow256$</td>
<td>$64\rightarrow256$</td>
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<td>Ceftriaxone</td>
<td>$&gt;256$</td>
<td>$&gt;256$</td>
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<tr>
<td>Meropenem</td>
<td>$8\rightarrow32$</td>
<td>$12\rightarrow32$</td>
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<td>Imipenem</td>
<td>$4\rightarrow32$</td>
<td>$8\rightarrow32$</td>
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<tr>
<td>Fosfomycin</td>
<td>$3\rightarrow8$</td>
<td>$8$</td>
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<tr>
<td>Gentamicin</td>
<td>$1,024\rightarrow2,048$</td>
<td>$1,024\rightarrow2,048$</td>
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<td>Tobramycin</td>
<td>$384\rightarrow1,024$</td>
<td>$256\rightarrow512$</td>
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<td>Colistin</td>
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<td>$0.125-0.38$</td>
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<td>Tigecycline</td>
<td>$1.5-3$</td>
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</tbody>
</table>

MICs were interpreted by using Clinical and Laboratory Standards Institute guidelines (www.clsi.org).
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