To the Editor: The \( \text{bla}_{\text{NDM-1}} \) gene, which produces the New Delhi metallo-\( \beta \)-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, \( \text{bla}_{\text{NDM-1}} \)-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 \( \text{bla}_{\text{NDM-1}} \) 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 \( \text{bla}_{\text{NDM-1}} \)-positive bacteria in an environmental setting in New Delhi was reported (6).

The possible appearance of bacteria harboring \( \text{bla}_{\text{NDM-1}} \) 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 \( \text{bla}_{\text{NDM-1}} \)-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 Transysem (CO-PAN 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 \( \times g \) for 30 seconds; 1 \( \mu \)L of the resulting suspension was then used as PCR template to detect \( \text{bla}_{\text{NDM-1}} \), as described (7). Two samples were positive for \( \text{bla}_{\text{NDM-1}} \); these 2 samples were collected from the same river (Kim Nguu River) but at sites 3 km apart.

To isolate and identify the phenotype and genotype of \( \text{bla}_{\text{NDM-1}} \)-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 \( \text{bla}_{\text{NDM-1}} \): Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. fluorescens/putida, and P. luteola. These isolates were placed onto media containing 0.5 mg/L meropenem, and bacterial DNA was extracted and used for the template for PCR analysis to detect \( \text{bla}_{\text{NDM-1}} \), as described (7). \( \text{bla}_{\text{NDM-1}} \) 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 \( \beta \)-lactam antimicrobial drugs, including carbapenems (Table). To
detect another β-lactamase, multiplex PCRs were carried out as described (8); genetic variants bla<sub>TEM</sub>, bla<sub>SHV</sub>, bla<sub>OXA</sub>, bla<sub>CTX-M</sub>, bla<sub>IMI</sub>, or bla<sub>VIM</sub> were not detected in any of the isolates other than <i>K. pneumoniae</i>. All 6 <i>K. pneumoniae</i> isolates were positive for bla<sub>TEM</sub> and bla<sub>CTX-M</sub> variants by PCR; these variants were confirmed as bla<sub>TEM</sub>-1 and bla<sub>CTX-M</sub>-3 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 <i>K. pneumoniae</i> isolates we obtained were also positive for <i>amr</i>, <i>arm</i>, and <i>gnt</i> genes encoding important β-lactamases. Multiplex PCR assays for the detection of <i>amr</i>, <i>arm</i>, and <i>gnt</i> genes encoding important β-lactamases were carried out as previously described (6), but we found no transconjugant strain with bla<sub>NDM-1</sub> on MacConkey agar containing 100 mg/L sodium azide and 0.5 mg/L meropenem.

Our results show that bla<sub>NDM-1</sub>-positive <i>K. pneumoniae</i> sequence type 283 is present in the Kim Nguu River, which flows through the central part of Hanoi at 2 sites. The isolates we obtained were also positive for 2 other β-lactamases, bla<sub>TEM-1</sub> and bla<sub>CTX-M-3</sub>, 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<sub>NDM-1</sub> are urgently needed.

### Table.

**Resistance to 13 antimicrobial drugs of bla<sub>NDM-1</sub>-positive Klebsiella pneumoniae isolates from the Kim Nguu River, Hanoi, Vietnam*<sup>†</sup>**

| Antimicrobial drug       | MIC, mg/L | Site X | Site Y |
|--------------------------|-----------|--------|--------|
| Piperacillin/tazobactam  | 64–256    | 64–256 |        |
| Cefotaxime               | 48–256    | 96–128 |        |
| Ceftriaxone              | >256      | >256   |        |
| Meropenem                | 8–32      | 12–32  |        |
| Doripenem                | 4–32      | 8–32   |        |
| Imipenem                 | 6–32      | >32    |        |
| Fosfomycin               | 3–8       | 8      |        |
| Gentamicin               | >1,024    | >1,024 |        |
| Tobramycin               | 384–1,024 | 256–384|        |
| Ciprofloxacin            | 0.064–1.5 | 0.064  |        |
| Colistin                 | 0.19–2    | 0.125–0.38| |
| Tetracycline             | 1.5–3     | 0.5–1.5|        |

*<sup>†</sup>MICs were interpreted by using Clinical and Laboratory Standards Institute guidelines (www.clsi.org).

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Rickettsia felis in Fleas, Southern Ethiopia, 2010

To the Editor: Fleas (order Siphonaptera) are obligate hematophagous insects. They are laterally flattened, holometabolous, and wingless ectoparasites. More than 2,500 species of flea, belonging to 16 families and 238 genera, have been described. A minority of these genera live in close association with humans (synanthropic), including fleas of these species: *Pulex irritans*, *Ctenocephalides felis*, *Ctenocephalides canis*, *Xenopsylla cheopis*, *Nosopsyllus fasciatus*, *Echidnophaga gallinacea*, and *Tunga penetrans* (1). Many fleas are capable of transmitting the following pathogens to their hosts: bacteria (e.g., *Rickettsia typhi*, *R. felis*, *Yersinia pestis*, and many *Bartonella* spp.); viruses (e.g., myxoma virus); protozoa (e.g., *Trypanosoma* spp.); or helminths (e.g., *Hymenolepis* spp.) (2). *Ctenocephalides* spp. fleas are of special interest as main reservoirs and vectors of *R. felis*, because this agent causes an emerging disease, fleaborne rickettsiosis. The distribution and prevalence of this disease have not been well studied. Symptoms of this disease range from mild to moderate and include fever, cutaneous rash, and sometimes an inoculation eschar. *R. felis* can also infect at least 10 other species of arthropods, including *P. irritans* fleas, trombiculid and mesostigmata mites, hard and soft ticks, and booklice (3,4).

In Africa, the presence of *R. felis* in fleas has been documented in Algeria, Tunisia, Egypt, Ethiopia, Gabon, Côte d’Ivoire, and the Democratic Republic of Congo (5). Recent studies conducted in Senegal (3) and Kenya (4) have shown that as much as 4.4% and 3.7%, respectively, of acute febrile diseases in these regions may be caused by *R. felis* infections. We conducted a study to determine the distribution and prevalence of *R. felis* in fleas in Ethiopia.

In our study, 55 fleas were collected in 2010 in 2 villages in Ethiopia; 25 fleas were collected from Tikemit Eshet (6°51’34″N and 35°51’34″E; altitude2,121 m), and 30 fleas were collected from Mizan Teferi (6°59’64″N and 35°35’50″E; altitude 1,700 m). The specimens were collected by using a plate filled with soapy water with a candle in the middle of the plate. Because fleas are thermotropic, they jumped toward the candle and fell onto the plate, where they rapidly drowned in the soapy water. The fleas were identified by morphologic features and stored in 90% ethanol until DNA extraction.

To confirm the phenotypic identification, we designed primers and probes for quantitative real-time PCR (qPCR) that were specific for 2 species of flea (*P. irritans* and *C. felis*) based on the sequences of mitochondrial cytochrome oxidase gene published in GenBank (Table). All of the identifications made by morphologic appearance were confirmed by qPCR because some specimens were damaged and difficult to identify. We found that most (52/55) of the fleas collected in human dwellings were *P. irritans*, and 3 specimens were *C. felis*. A screening by amplification using primers and probes specific for the 16S–23S internal transcribed spacer of *Bartonella* spp. (7) produced no positive results.

We screened rickettsial DNA by using qPCR with a *Rickettsia*-specific, *gltA* gene-based RKND03 system (8) and a *bioB*-based qPCR system specific for *R. felis*. We found that the 3 specimens of *C. felis* fleas contained the DNA of *R. felis*; however, 23 (43%) of 53 *P. irritans* specimens also contained DNA of *R. felis*. We amplified and sequenced nearly the entire rickettsial *gltA* gene from 3 *C. felis* and 10 *P. irritans* specimens and found that the sequence was identical to that of *R. felis* (GenBank accession no. NC_0071111).

During the field collection of the fleas, the conservation of specimens may be difficult. Degradation of specimens may pose a problem for the ensuing morphologic identification. For fleas, a specific preparation is required that destroys internal organs and produces a chitin complex of the insect. This type of preparation makes it difficult, and sometimes impossible, to use the insect later for molecular studies. The development of qPCR specific for *P. irritans* and *C. felis* fleas facilitated the identification of damaged samples and also precluded the laborious and time-consuming procedure of identification by morphologic features.

We conclude that the reservoirs of *R. felis* in Ethiopia include both *C. felis* and *P. irritans* fleas. In Ethiopia, *P. irritans* fleas have been reported to be prevalent (9). *P. irritans* fleas have been shown to be infected by *R. felis* in several locations, notably in the Democratic Republic of the

| Species | Forward primer, 3′→5′ | Reverse primer, 3′→5′ | Fluorescent probe |
|---------|-----------------------|-----------------------|------------------|
| *P. irritans* | CCAATACTTTTAGAAGGACCAAAAAACAGATTGATGGACAAATAGATTTAGATGCGATTT | TTGGCTTACGGCTTCTTCTCGACTTTTT | TCCGTTAATCTTTTTT
| *C. felis* | TCGTTATTTACTGGAAGGACCAAAAAATGCATTGATGGACAAATAGATTTAGATGCGATTT | TTGGCTTACGGCTTCTTCTCGACTTTTT | TCCGTTAATCTTTTTT

*P.* *Pulex; C.* *Ctenocephalides.*