4. Chung KP, Tseng SP, Huang YT, Tsai TH, Teng LJ, Hsueh PR. Arrival of *Klebsiella pneumoniae* carbapenemase (KPC)-2 in Taiwan. J Antimicrob Chemother. 2011;66:1182–4. Epub 2011 Feb 8. http://dx.doi.org/10.1093/jac/dkr025
5. Centers for Disease Control and Prevention. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* in acute care facilities. MMWR Morb Mortal Wkly Rep. 2009;58:256–60.
6. Tacconelli E, Karchmer AW, Yokoe D, D'Agata EMC. Preventing the influx of vancomycin-resistant enterococci into health care institutions, by a simple validated prediction rule. Clin Infect Dis. 2004;39:964–70. http://dx.doi.org/10.1086/423961
7. Balm MN, Ngan G, Jureen R, Lin RT, Teo J. Molecular characterization of newly emerged bla_{KPC-2}–producing *Klebsiella pneumoniae* in Singapore. J Clin Microbiol. 2012;50:475–6. Epub 2011 Nov 23. http://dx.doi.org/10.1128/JCM.05914-11
8. Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. J Antimicrob Chemother. 2011;66:307–12. http://dx.doi.org/10.1093/jac/dkq431
9. Hsu LY, Tan TY, Tam VH, Kwa A, Fisher DA, Koh TH, et al. Surveillance and correlation of antibiotic prescription and resistance of gram-negative bacteria in Singaporean hospitals. Antimicrob Agents Chemother. 2010;54:1173–8. http://dx.doi.org/10.1128/AAC.01076-09
10. Urban C, Bradford PA, Tuckman M, Segal-Maurer S, Wehbeh W, Grenner L, et al. Carbapenem-resistant *Escherichia coli* harboring *Klebsiella pneumoniae* carbapenemase β-lactamases associated with long-term care facilities. Clin Infect Dis. 2008;46:e127–30. http://dx.doi.org/10.1086/588048

Address for correspondence: Indumathi Venkatachalam, National University Hospital–Infectious Diseases, 5 Lower Kent Ridge Rd, Singapore City 119074, Singapore; email: indumathi_venkatachalam@nuhs.edu.sg

bla_{NDM-1}–positive *Klebsiella pneumoniae* from Environment, Vietnam

To the Editor: The bla_{NDM-1} 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_{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 bla_{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 bla_{NDM-1}–positive bacteria in an environmental setting in New Delhi was reported (*6*).

The possible appearance of bacteria harboring bla_{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 bla_{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 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_{NDM-1} as described (*7*). Two samples were positive for bla_{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 bla_{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 bla_{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 bla_{NDM-1} as described (*7*). bla_{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 β-lactam antimicrobial drugs, including carbapenems (Table). To

Table. Resistance to 13 antimicrobial drugs of bla_{NDM-1}–positive *Klebsiella pneumoniae* isolates from the Kim Nguu River, Hanoi, Vietnam*

Antimicrobial drug	MIC, mg/L	
	Site X	Site Y
Piperacillin/tazobactam	64–>256	64–>256
Cefotaxime	48–>256	96–128
Ceftazidime	>256	>256
Ceftriaxone	96–>256	128–>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
Tgecycline	1.5–3	0.5–1.5

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

detect another β-lactamase, multiplex PCRs were carried out as described (*8*); genetic variants bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CTX-M}, bla_{IMP}, bla_{VIM}, and bla_{KPC} 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-1} and $bla_{CTX-M-3}$ 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 *K. pneumoniae* isolates we found were highly resistant to gentamicin (MIC >1,024 mg/L) and tobramycin (MIC 256–>1,024 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-1}. The azide-resistant *Escherichia coli* strain J53 has been used as recipient for conjugation assay, which had been reported previously (*6*), but we found no transconjugant strain with bla_{NDM-1} on MacConkey agar containing 100 mg/L sodium azide and 0.5 mg/L meropenem.

Our results show that bla_{NDM-1}–positive *K. pneumoniae* 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_{TEM-1} and $bla_{CTX-M-3}$, 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-1} are urgently needed.

Rie Isozumi, Kumiko Yoshimatsu, Tetsu Yamashiro, Futoshi Hasebe, Binh Minh Nguyen, Tuan Cuong Ngo, Shumpei P. Yasuda, Takaaki Koma, Kenta Shimizu, and Jiro Arikawa

Author affiliations: Hokkaido University, Sapporo, Japan (R. Isozumi, K. Yoshimatsu, S.P. Yasuda, T. Koma, K. Shimizu, J. Arikawa); Nagasaki University, Nagasaki, Japan (T. Yamashiro, F. Hasebe); and National Institute of Hygiene and Epidemiology, Hanoi, Vietnam (B.M.. Nguyen, T. C. Ngo)

DOI: http://dx.doi.org/10.3201/eid1808.111816

References

1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, *bla* (NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. http://dx.doi.org/10.1128/AAC.00774-09
2. Nordmann P, Nass T, Poirel L. Global spread of carbapenem-producing *Enterobacteriaceae*. Emerg Infect Dis. 2011;17:1791–8.
3. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2
4. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010;36:S8–14. http://dx.doi.org/10.1016/S0924-8579(10)70004-2
5. Moellering RC Jr. NDM-1—a cause for worldwide concern. N Engl J Med. 2010;363:2377–9. http://dx.doi.org/10.1056/NEJMp1011715
6. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 2011;11:355–62. http://dx.doi.org/10.1016/S1473-3099(11)70059-7
7. Pfeifer Y, Wilharm G, Zander E, Wichelhaus TA, Gottig S, Hunfeld KP, et al. Molecular characterization of bla_{NDM-1} in an *Acinetobacter baumannii* strain isolated in Germany in 2007. J Antimicrob Chemother. 2011;66:1998–2001. http://dx.doi.org/10.1093/jac/dkr256
8. Dallenne C, Da Costa A, Decre D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother. 2010;65:490–5. http://dx.doi.org/10.1093/jac/dkp498
9. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis. 2007;45:88–94. http://dx.doi.org/10.1086/518605
10. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol. 2005;43:4178–82. http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005

Address for correspondence: Rie Isozumi, Hokkaido University, Kita15, Nishi7, Kita-ku, Sapporo, Hokkaido, 060-8638, Japan; email: wushiqilihui@hotmail.co.jp

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 (*3*,*4*). *R. felis* can also infect at least 10 other species of arthropods, including *P. irritans* fleas, trombiculid and mesostygmata mites, hard and soft ticks, and booklice (*5*,*6*).

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′837″N and 35°51′348″E; altitude2,121 m), and 30 fleas were collected from Mizan Teferi (6°59′640″N and 35°35′507″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_007111).

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

Table. Sequences of primers and probes used to identify fleas by quantitative real-time PCR, southern Ethiopia, 2010

Species	Forward primer, 3′→5′	Reverse primer, 3′→5′	Fluorescent probe
P. irritans	CGAATACTTTTAGAAAGCCAAAACA	CATTGATGACCAATAGATTTTAGAGTG	TTGCTTTACCGTCTTTACGTTT
C. felis	TCGTTATTTACTTGAAAGACAAAATG	TCATTGATGACCAATTGCTTT	TGCTTTACCTTCTCTTCGACTTTT

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