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Klebsiella pneumoniae Carbapenemase-producing Enterobacteria in Hospital, Singapore

To the Editor: During the past decade, enterobacteria that produce *Klebsiella pneumoniae* carbapenemase (KPC) have become established in the United States and countries in South America and Europe (*1*). In Asia, KPC was reported in the People's Republic of China in 2007 (*2*) and subsequently in South Korea (*3*) and Taiwan (*4*). Public health agencies emphasize screening and strict contact precautions to control multidrug resistant *Enterobacteriaceae* (*5*). Routine testing for mechanisms of resistance facilitates detection of emerging carbapenem-resistant *Enterobacteriaceae*.

In Singapore's 1,000-bed National University Hospital during November 2010–January 2011, we identified New Delhi metallo-β-lactamase 1–producing *Enterobacteriaceae* in 2 clinical specimens but none that produced KPC (I. Venkatachalam et al., unpub. data). We conducted a laboratory screening study to determine the prevalence and nature of carbapenem-resistant *Enterobacteriaceae* in April 2011. Ethics committee approval was waived for this study.

Testing of rectal swab samples is part of an established hospitalwide program for vancomycin-resistant enterococci screening. Using a scoring system to identify patients at high risk for vancomycin-resistant enterococci (*6*), we found that ≈2.5 specimens per 100 admissions were attained each month. During our study, we also tested these samples for carbapenemase-producing *Enterobacteriaceae*.

During April–June 2011, we incubated specimens for 24 h in 10 mL tryptic soy broth containing 1 mg/L imipenem, then streaked 100 μL of the broth onto CHROMagar KPC (CHROMagar, Paris, France). Colonies detected after 24 h incubation at 35°C were identified by using MALDI-TOF MS with a Microflex LT instrument (Bruker Daltonik GmbH, Leipzig, Germany). Imipenem and meropenem MICs for *Enterobacteriacaeae* were confirmed by using Etests (bioMérieux, Marcy l'Etoile, France). Isolates with MIC ≥2 μg/mL underwent analysis with Metallo-β-Lactamase Confirmative Identification Pack (Rosco Diagnostica, Taastrup, Denmark) and Etest MBL (bioMérieux) for metallo-β-lactamase production. Isolates suspected to be producers were genotypically confirmed by PCR.

Of the 201 nonduplicate samples processed, 79 microorganisms exhibited imipenem resistance and were isolated on CHROMagar KPC (Table). Among *Enterobacteriaceae*, carbapenem MIC ≥2 μg/mL was present in 1 *E. aerogenes,* 2 *E. cloacae*, and 4 *K. pneumoniae* isolates. One isolate (*K. pneumoniae*) had a positive combined disk test result with a pattern suggestive of serine carbapenemase production.

We analyzed genomic DNA (DNeasy Blood and Tissue Kit, QIAGEN, Hilden, Germany) from this isolate by using PCR for transmissible carbapenem resistance markers: metallo-β-lactamases (VIM, IMP, and KHM-1), serine carbapenemases (KPC, GES1–5 and 7), and OXA-48. bla_{KPC}-specific primers (forward primer 5′-CGTTGACGCCCAATCC-3′; reverse primer 5′-ACCGCTGGCAGCTGG-3′) generated a 390-bp amplicon. Full gene sequencing of bla_{KPC} (forward primer 5′-ATGTCACTGTATCGCCGTCT-3′; reverse primer 5′-CCTAAATGTGACAGTGGTGG) revealed 100% homology to bla_{KPC-2} (GenBank accession no. FJ628167.2). Further analysis showed that the isolate carried extended-

spectrum β-lactamase (bla_{TEM-1}, bla_{SHV-11}, $bla_{CTX-M-15}$), plasmid-located AmpC (bla_{DHA-1}), and 16S rRNA methylase *armA* genes but was negative for bla_{CMY}, bla_{OXA}, bla_{GES}, metallo-β-lactamases, and plasmid-mediated quinolone (*qnr*) genes. Multilocus sequence typing conducted at Institut Pasteur (Paris, France), identified this isolate as sequence type 11. It was susceptible only to colistin and tigecycline.

Sequence type 11, a single-locus variant of the internationally dominant sequence type 258 clone (*7*), is present in 64.2% of KPC-producing *K. pneumoniae* in China (*8*). In South Korea, sequence type 11 is the most common clone of extended-spectrum β-lactamase–producing *K. pneumoniae* isolates (*3*).

The KPC-producing *K. pneumoniae* originated from a woman in the local community, 89 years of age, who had severe ischemic cardiomyopathy and atrial fibrillation. She was discharged home after a 3-day hospitalization for treatment of stroke in January 2011. During May 2011, she was readmitted after a severe stroke. During week 4, she was transferred to a subacute care hospital but readmitted within 24 hours with a lower respiratory tract infection. A rectal swab sample was collected for routine screening for vancomycin-resistant enterococci.

We empirically prescribed a 10-day course of piperacillin-tazobactam. On day 10 of treatment, KPC-producing *K. pneumoniae* was isolated from the rectal specimen. The patient responded to treatment and was discharged to a long-term care facility.

This case demonstrates concerns about a KPC of local community origin because no other KPC-producing *Enterobacteriaceae* were isolated during this inpatient surveillance and the patient had neither received antimicrobial drugs nor traveled in the 6 months before her May admission (*7*). However, she was admitted 3 weeks before sampling; an unidentified hospital source remains a possibility. Of added concern is the potential for dissemination within the facility to which she was discharged.

Resistance to third-generation cephalosporins was reported for 20% of *Escherichia coli*, 32.3% of *K. pneumoniae*, 46.2% of *Acinetobacter* spp., and 7.5% of *Pseudomonas aeruginosa* clinical isolates at 4 major Singapore hospitals during January 2006–December 2008 (*9*). Authors reported positive correlation between meropenem administration and carbapenem resistance development in *Acinetobacter* spp. blood isolates.

When the resistance mechanism to an antimicrobial drug is embedded in highly mobile elements like plasmids, widespread dissemination is possible. Although acute care hospitals are conducive to development of antimicrobial drug resistance, long-term care facilities facilitate spread of these organisms (*10*). Infection control interventions including routine screening for mechanisms of resistance and responsible use of antimicrobial drugs are increasingly critical in hospitals and long-term care facilities; a response plan coordinated between these facilities is needed.

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Table. Bacteria isolated on CHROMagar in screening for carbapenemase-producing *Enterobacteriaceae*, Singapore, 2011*

Organism	No. isolated
Enterobacteriaceae	
Klebsiella pneumoniae	11
Enterobacter cloacae	3
Enterobacter aerogenes	2
Proteus mirabilis	2
Escherichia coli	1
Serratia marcescens	1
Non-*Enterobacteriaceae*: gram-negative nonfermenters	
Pseudomonas aeruginosa	20
Acinetobacter baumannii	17
Stenotrophomonas maltophilia	13
Elizabethkingia meningoseptica	3
Wautersiella falsenii	1
Enterococci	
Enterococcus gallinarum	4
Enterococcus faecalis	1

*CHROMagar, Paris, France.

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*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 (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 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