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NDM-1-producing Strains, Family Enterobacteriaceae, in Hospital, Beijing, China

To the Editor: The prevalence of New Delhi metallo- β -lactamase-1 (NDM-1)-producing strains (family *Enterobacteriaceae*) in China remains unclear. Recently, to clarify the prevalence of *bla*_{NDM-1} in *Enterobacteriaceae* strains, we carried out retrospective surveillance for *bla*_{NDM-1} among carbapenem-resistant enterobacterial strains isolated from patients at the Chinese PLA General Hospital in Beijing. This tertiary teaching hospital has 4,000 beds and 12,000 daily outpatient visits. More than 50% of patients admitted to the hospital are from areas outside Beijing. During January 2009–June 2013, a total of 8,586 enterobacterial isolates were obtained from routine clinical samples that had been passively sent to the microbiology department. Of these, 242 (2.8%) strains exhibited resistance to carbapenems.

In this study, we used PCR amplification to screen the carbapenem-resistant strains for the *bla*_{NDM-1} gene and other common resistance determinants. The MICs of various antimicrobial drugs were measured by E-test (AB bioMérieux, Solna, Sweden). S1 nuclease pulsed-field gel electrophoresis and Southern blot analysis were used to identify the sizes of *bla*_{NDM-1}-carrying plasmids. The incompatibility (Inc) groups of the plasmids were detected by several multiplex and simplex PCRs. Multi-locus sequence typing (MLST) was carried out for *Klebsiella pneumoniae* and *Escherichia coli* isolates, according to protocols provided on MLST websites (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html and <http://mlst.ucc.ie/mlst/dbs/Ecoli>). The transferability of plasmids was identified by conjugation experiments.

Five *bla*_{NDM-1}-positive enterobacterial isolates of the following species were identified: *E. coli* (1 isolate in October 2010), *K. pneumoniae* (1 isolate in August 2012), *Providencia rettgeri* (1 isolate in October 2012), *Enterobacter cloacae* (1 isolate in November 2012), and *Raoultella ornithinolytica* (1 isolate in March 2013). According to the 2013 Clinical and Laboratory Standards Institute performance standard M100-S23 (www.clsi.org), the NDM-1-producing *K. pneumoniae* (IR5047) isolate exhibited low-level resistance to imipenem and meropenem, whereas other isolates showed high-level resistance to carbapenems. Only *E. coli* and *Providencia rettgeri*, which carry 16S rRNA methylase genes, exhibited high-level resistance to amikacin (Table). S1 nuclease pulsed-field gel electrophoresis and Southern blot analysis showed that the *bla*_{NDM-1} gene was located on plasmids of various sizes belonging to different Inc groups. The *K. pneumoniae* isolate was defined as a novel ST1240 with the allelic profile 2–1–1–1–1–3–24, and the *E. coli* isolate was identified as ST167.

In China, various *bla*_{NDM-1}-carrying strains of the *Enterobacteriaceae* have been sporadically identified, including *K. pneumoniae*, *K. oxytoca*, *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Citrobacter freundii* (1–4). We identified a *P. rettgeri* isolate and an *R. ornithinolytica* isolate that produced NDM-1. The *bla*_{NDM-1}-positive *P. rettgeri* isolates have also been identified in Pakistan, India, Canada, and Mexico, whereas the NDM-1-producing *R. ornithinolytica* strain has only been detected in India (5–9). In this study, all 5 NDM-1-producing strains were isolated only once, and no dissemination of NDM-1-producing strains of *Enterobacteriaceae* has been found. Two strains (*K. pneumoniae* and *Enterobacter cloacae*) were isolated within 48 hours of the patient's hospital admission,

Table. Phenotype and molecular characteristics of NDM-1-producing strains isolated from Chinese PLA General Hospital, Beijing, China, 2009–2013*

Isolate no.	Organism	Source	ST†	<i>bla</i> _{NDM-1} plasmid size, type	Prevalence of RDs	MICs, mg/L							
						CTX	FEP	TZP	IPM	MEM	ETP	AK	LVX
IR5028	<i>Escherichia coli</i>	Urine	167	≈190 kb, A/C	<i>bla</i> _{TEM} , <i>bla</i> _{OXA-1} , ‡ <i>bla</i> _{CTX-group 1, 2, 9} , <i>aac(6′)-Ib-cr</i> , ‡ <i>armA</i> ‡	>256	>256	>256	>32	>32	>32	>256	>32.0
IR5047	<i>Klebsiella pneumoniae</i>	Blood	1,240	≈50 kb, X3	<i>bla</i> _{TEM} , <i>bla</i> _{SHV} , <i>bla</i> _{OXA-1} , <i>qnrS</i> , ‡ <i>oqxAB</i> ‡	>256	64	>256	8	6	>32	2	0.38
IR5337	<i>Providencia rettgeri</i>	Urine	NA‡	≈190 kb, A/C	<i>bla</i> _{PER} , <i>bla</i> _{CMY} , <i>mtC</i> ‡	>256	>256	>256	>32	>32	4	>256	>32.0
IR5338	<i>Enterobacter cloacae</i>	Urine	NA	≈50 kb, X3	<i>bla</i> _{SHV} , ‡ <i>bla</i> _{OXA-1} , <i>bla</i> _{CTX-group 2} <i>qnrS</i> ‡	>256	>256	>256	>32	>32	>32	1.5	2.0
IR5343	<i>Raoultella ornithinolytica</i>	Abscess	NA	≈70 kb, N	<i>qnrS</i> ‡	>256	32	>256	>32	>32	>32	2	0.75

*NDM-1, New Delhi metallo-β-lactamase-1-producing; ST, sequence type; RDs, resistance determinants; CTX, cefotaxime; FEP, cefepime; TZP, piperacillin-tazobactam; IMP, imipenem; MEM, meropenem; ETP, ertapenem; AK, amikacin; LVX, levofloxacin; NA, not applicable.

†The alleles at each of the multi-locus ST loci for a given isolate are combined into an allelic profile and assigned an ST designation.

‡Shown to be transferred by conjugation experiments.

indicating the infections were imported (from Shandong and Hebei Provinces, respectively). *Escherichia coli*, *P. rettgeri*, and *R. ornithinolytica* were isolated 48 hours after admission of patients (from Henan and Hebei Provinces). Therefore, the patients might have acquired the NDM-1-producing *Enterobacteriaceae* strains at the hospital.

However, the source of the *bla*_{NDM-1} determinant remains unclear. The possibility that the strains were imported cannot be excluded for the several reasons. First, examination to determine the infectious agent had not been performed for a considerable number of patients within 48 hours of their admission. Second, NDM-1-producing *Enterobacteriaceae* species have not spread in this hospital. Third, the *bla*_{NDM-1}-carrying plasmids in the same Inc group and of similar size exhibited substantial differences in resistance determinants (Table), which suggests a different evolutionary origin for these isolates. In an additional survey of clinical data, we found no epidemiologic relationship between the patients who were infected by NDM-1-producing pathogens. These data suggest a sporadic pattern of NDM-1-producing enterobacteria in the hospital.

Sequencing analysis (data not shown) indicated that the *bla*_{NDM-1}-carrying plasmid carried by *K. pneumoniae*

(≈50 kb, IncX3) was different from the plasmid found in the *Acinetobacter pittii* isolate that was disseminated in an intensive care unit of the Chinese PLA General Hospital in 2008 (10). This finding suggests that the 2 plasmids had a different evolutionary origin. The IncX3 plasmid that we found was highly homologous (>99%) to the plasmid pNDM-HN380 (GenBank accession no. JX104760), which has been identified in several *Enterobacteriaceae* strains isolated from patients in southern China (1). This finding showed that the IncX3 plasmid that was 50 kb in size acted as the main factor mediating the transmission of the *bla*_{NDM-1} gene across China. IncA/C plasmids are the leading group of *bla*_{NDM-1}-carrying plasmids and have been detected in *E. coli* isolated from China (1). In this study, *E. coli* (IR5028) and *P. rettgeri* (IR5337) carried IncA/C plasmids. However, these 2 strains exhibited diverse resistant determinants on these plasmids (Table). This observation suggested that the 2 plasmids have different integrating processes. For *R. ornithinolytica*, the *bla*_{NDM-1} gene was located on an IncN plasmid of ≈70 kb, which is very different from other plasmids.

In conclusion, we identified various NDM-1-producing enterobacterial isolates at the Chinese PLA Hospital in Beijing and the emergence of novel *bla*_{NDM-1}-carrying clones among

common species of *Enterobacteriaceae*, such as *K. pneumoniae* ST1240 and *E. coli* ST167. There is an urgent need for monitoring and surveillance of epidemiologic and genotypic profiles of NDM-1-producing *Enterobacteriaceae* species in China.

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