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LETTER TO THE EDITOR

NDM-4- and NDM-5-Producing *Klebsiella pneumoniae* Coinfection in a 6-Month-Old Infant

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Carbapenemase-producing *Enterobacteriaceae* are a serious problem worldwide (1). Among the newly emerging carbapenemases, New Delhi metallo-β-lactamase (NDM) is one of the antimicrobial resistance factors causing greatest concern because its global spread has been rapid and it is frequently associated with other resistance genes (2). Sixteen variant NDM enzymes have been discovered in different countries since the identification of NDM-1, all of which are cataloged at http://www.lahey.org/Studies/other.asp. NDM-4 and NDM-5 differ from NDM-1 by one and two amino acid substitutions, respectively, and are associated with increased carbapenemase activity (2, 3). Here, we report for the first time the cooccurrence of NDM-4 and NDM-5 producers in the same patient, NDM-4- and NDM-5-producing *Klebsiella pneumoniae* in the Middle East, and bla_{NDM-4} in *K. pneumoniae*.

A 6-month-old infant was hospitalized in the Kafr El-Sheikh City, Egypt, public hospital suffering from tachypnea, tachycardia, and pneumonia due to transposition of the great arteries with atrial septostomy and intracranial hemorrhage in April 2014. Ten days after his admission, *K. pneumoniae* KPE127 was recovered from an endotracheal tube swab. After another 10 days, the patient suffered septicemia, and *K. pneumoniae* KPB140 was recovered from the blood. The patient was hospitalized for about 70 days and received prolonged courses of broad-spectrum antibiotics, including vancomycin, imipenem, ceftazidime, ceftriaxone, and amikacin. *K. pneumoniae* KPE127 and *K. pneumoniae* KPB140 were resistant to all β-lactam antibiotics tested, gentamicin, tetracycline, nalidixic acid, and norfloxacin (Table 1). Phenotypic carbapenemase production was confirmed in both strains with a modified Hodge test and carbapenem inactivation method (4); metallo-β-lactamase production was also confirmed with sodium mercaptoacetic acid. The presence of carbapenemases and other β-lactamases was determined with PCR and DNA sequencing. *K. pneumoniae* KPE127 harbored bla_{NDM-4}, $bla_{CTX-M-15}$, $bla_{SHV-142}$, bla_{TEM-1}, *qnrS*, *aac(6′)-Ib-cr*, and two different types of class 1 integrons (*dfrA12-aadA2* and *aadA2*), whereas *K. pneumoniae* KPB140 carried bla_{NDM-5}, $bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV-1}, and a class 1 integron (*dfrA12-aadA2*).

The genetic environment of the bla_{NDM-4} and bla_{NDM-5} genes was analyzed with PCR mapping, as previously described (5). The insertion sequence, IS*Aba125*, was identified upstream and the bleomycin resistance gene, ble_{MBL}, downstream of both the bla_{NDM-4} and bla_{NDM-5} genes. Therefore, the genetic environment of bla_{NDM-4} and bla_{NDM-5} was similar to that previously described in most NDM-1-positive enterobacterial isolates (5).

The transferability of the bla_{NDM-4} and bla_{NDM-5} genes was studied by conjugation experiments with an *Escherichia coli* J53 recipient (5). However, after we attempted multiple times to perform conjugation experiments, bla_{NDM-4} and bla_{NDM-5} could not be transferred, indicating their potential locations on a nonconjugative plasmid. Plasmid analysis and Southern blotting confirmed that the bla_{NDM-4} and bla_{NDM-5} genes were located on a two different plasmids of >93 kb in size. The PCR-based replicon typing method was used to identify the incompatibility group of bla_{NDM-4}- and bla_{NDM-5}-positive plasmids as previously reported (6). The results revealed that the bla_{NDM-4}-positive plasmid belongs to the L/M incompatibility group and that the bla_{NDM-5}-positive plasmid is nontypeable. IncL/M plasmids were previously identified from different environmental and clinical isolates and are considered an emerging threat, since this group of plasmids has increasingly been identified as a source of different β-lactamases, including the metallo-β-lactamase NDM-1, the class D carbapenemase OXA-48, and the extended-spectrum β-lactamase $bla_{CTX-M-3}$ (7).

Multilocus sequence typing (MLST) was performed according to the *Klebsiella* sequence typing website (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). MLST analysis revealed that both strains belong to sequence type 45 (ST45). Enterobacterial repetitive intergenic consensus (ERIC) sequence PCR analysis of both isolates showed different patterns, indicating their genetic diversity (data not shown). ST45 corresponds to the ST of the most recently identified NDM-5-positive *K. pneumoniae* strain identified in Australia (8) and the ST of the NDM-1-positive *K. pneumoniae* strain identified in Turkey (9). However, it does not correspond to the ST of the NDM-5-positive *K. pneumoniae* strain, which was ST147, from a patient transferred from the United Arab Emirates to South Korea (2). It has previously been suggested that the Middle East, which is geographically close to the Indian subcontinent, is another reservoir of NDM-producing *Enterobacteriaceae* (3). Our findings support this proposition, because neither patient had a history of travel, suggesting that NDM-4 and NDM-5 were hospital acquired and autochthonous in Egypt.

Our study confirms the emergence of NDM-4- and NDM-5-producing bacteria in the African continent after their recent

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TABLE 1 Susceptibilities of the NDM-4- and NDM-5-producing *Klebsiella pneumoniae* strains

Strain	MIC (μg/ml)[a]											
	IMP	MPM	AZT	AMX	CTX	CAZ	CFP	CRO	GEN	NAL	TET	NOR
K. pneumoniae KPE127	>32	>32	>512	>512	>512	>512	>512	512	128	512	512	128
K. pneumoniae KPB140	>32	>32	>512	>512	512	>512	>512	512	128	512	512	128

[a] IMP, imipenem; MPM, meropenem; AZT, aztreonam; AMX, amoxicillin; CTX, cefotaxime; CAZ, ceftazidime; CFP, cefoperazone; CRO, ceftriaxone; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline; NOR, norfloxacin.

identification in Cameroon and Algeria, respectively (3, 10). The worldwide emergence of NDM-4 and NDM-5 producers is quite alarming, particularly when the increased carbapenemase activity of NDM-4 and NDM-5, compared with that of NDM-1, is taken into account. Moreover, the cooccurrence of two highly resistant bacteria in the same patient is extremely worrisome because it might lead to therapeutic failure and death. Therefore, it is imperative that the relevant medical authorities consider the prevalence of NDM enzymes within the community to monitor future trends and prevent spread into epidemic clonal lineages.

Nucleotide sequence accession numbers. The nucleotide sequences of the bla_{NDM-4}- and bla_{NDM-5}-containing *K. pneumoniae* strains have been deposited in the DDBJ/GenBank/EMBL database under accession no. LC145700 and LC146472, respectively.

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