Emergence of *Klebsiella pneumoniae* ST273 Carrying *bla*$_{NDM-7}$ and ST656 Carrying *bla*$_{NDM-1}$ in Manila, Philippines

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We sought to determine the epidemiology of carbapenem-resistant *Enterobacteriaceae* and to investigate the emergence of carbapenem-resistant *Klebsiella pneumoniae* in two teaching hospitals in Manila, Philippines. We screened 364 *Enterobacteriaceae* for carbapenem resistance between 2012 and 2013 and detected four carbapenem-resistant *K. pneumoniae* isolates from three different patients. We used whole genome sequencing to determine the antibiotic resistance profiles and confirmed the presence of carbapenemase genes by multiplex PCR. We used multilocus sequence typing and PCR-based replicon typing to genetically characterize the carbapenem-resistant isolates. The carbapenemase gene *bla*$_{NDM}$ was detected in *K. pneumoniae* isolates from two patients. The first patient had ventilator-associated pneumonia and lumbar shunt infection from *K. pneumoniae* ST273 carrying *bla*$_{NDM-7}$. The second patient had asymptomatic genitourinary colonization with *K. pneumoniae* ST656 carrying *bla*$_{NDM-1}$. The third patient had a gluteal abscess with *K. pneumoniae* ST1 that did not carry a carbapenemase gene, but did carry *bla*$_{DHA-1}$, *bla*$_{OXA-1}$, and *bla*$_{SHV-1}$.

In this study, we report the first cases of *bla*$_{NDM}$-carrying pathogens in the Philippines and add to the growing evidence of the worldwide spread of ST273 and NDM-7, a more efficient carbapenem hydrolyzer than NDM-1.

**Keywords:** metallo-beta-lactamase, carbapenemase, carbapenem resistant, molecular epidemiology, *Enterobacteriaceae*, whole genome sequencing

**Introduction**

**New-Delhi metallo-beta-lactamase-1** (NDM-1) is the most recently described metallo-beta-lactamase and has emerged as a global health threat.1 Similar to other metallo-beta-lactamas, NDM-1 can hydrolyze all beta-lactams except aztreonam. NDM-1 is a global health threat because the gene encoding NDM-1, *bla*$_{NDM-1}$, is found on more diverse mobile genetic elements than other metallo-beta-lactamase genes.2 Since first detected in 2008, NDM-1 has been reported on every continent except the Antarctica, although the main reservoirs appear to be the Indian subcontinent, the Balkan region, and the Middle East.3,4 NDM-1 has been sporadically detected in Southeast Asia, but never in the Philippines, where previous regional surveillance of carbapenemases detected IMP-26 only.5,6 Of 24,684 isolates analyzed by the Philippines Department of Health, only 0.7% of *Klebsiella* were carbapenem resistant, although carbapenemase gene testing was not reported.7 During passive surveillance of antimicrobial resistance in two academic teaching hospitals, we identified the emergence of carbapenem-resistant *K. pneumoniae* and sought to determine which beta-lactamase and carbapenemase genes were present in these isolates. In this study, we report the detection of *bla*$_{NDM-1}$ and *bla*$_{NDM-7}$, which produces a more efficient carbapenem hydrolyzer than NDM-1, and the spread of *K. pneumoniae* ST273, a clone with outbreak and epidemic potential.

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**Materials and Methods**

**Microbial identification and antibiotic susceptibility testing**

Collection of organisms was from January 2012 to February 2013 at two affiliated academic teaching hospitals in metropolitan Manila, Philippines. Organism identification and antibiotic minimum inhibitory concentration (MICs) were determined by the VITEK-2 system using VITEK card AST-N261 (bioMérieux, Paris, France) (Table 1). Carbapenemase activity was detected by the modified Hodge test. Microbiological tests were performed and interpreted according to the procedures of the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement M100-S21 (2011).

**Carbapenemase gene detection**

Carbapenemase genes were amplified using a previously described multiplex PCR. PCR products were sequenced by the Sanger method (Lone Star Labs, Houston, TX) and from the Lahey Clinic database of β-lactamases (http://lahey.org/studies/).4,8,9 Primers, other reagents, and the thermocycling settings were as previously described.11

**Whole genome sequencing**

DNA was extracted with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Sequencing was performed on an Illumina MiSeq platform and underwent de novo assembly. Beta-lactamase genes were detected using ResFinder 2.1.12

**Results**

**Detection of carbapenem-resistant Enterobacteriaceae**

Three hundred sixty-four Enterobacteriaceae were collected, including 181 Escherichia coli, 135 Klebsiella spp., 19 Enterobacter spp., and 38 other Enterobacteriaceae. Four Klebsiella pneumoniae isolates from three unique patients were resistant to at least one carbapenem (ertapenem, imipenem, or meropenem). All carbapenem-resistant K. pneumoniae were detected during the same month and were investigated as a possible outbreak. One isolate (ARPG-315) displayed low-level meropenem resistance (MIC to meropenem of 2 mg/ml). Three K. pneumoniae isolates (designated as ARPG-379, ARPG-383, and ARP-664) had high-level meropenem resistance (≥16 mg/ml).

**Clinical histories of patients with carbapenem-resistant K. pneumoniae infections**

Patient 1 was a 70-year-old woman transferred to St. Luke’s Medical Center Global City in 2013 because of a subarachnoid hemorrhage requiring intubation and placement of a lumbar drain. After transfer, on hospital day 3, she developed ventilator-associated pneumonia caused by a carbapenem-susceptible...
K. pneumoniae (ARPG-318) and was treated with meropenem. On day 33, the patient developed a lumbar shunt infection and ventilator-associated pneumonia from a carbapenem-resistant K. pneumoniae (ARPG-379 and ARPG-383). The patient’s treatment was escalated to include colistin and amikacin, which resolved the infection. On day 66, the patient had an acute neurological decline and was discharged from the hospital in poor neurological condition at the family’s request.

Patient 2 was a 92-year-old man admitted to St. Luke’s Medical Center Quezon City in 2013 for a gastrostomy tube exchange. Postoperative course was complicated by respiratory failure and ventilator-associated pneumonia caused by Pseudomonas aeruginosa, which was treated with meropenem. On day 21, a urine culture grew carbapenem-resistant K. pneumoniae (ARP-664). The culture was interpreted as colonization, and no antimicrobial agent was administered. No active genitourinary infections occurred, but on day 46, the patient’s respiratory function worsened and the patient died.

Patient 3 was a 23-year-old man admitted to St. Luke’s Medical Center Global City in 2013 with acute lymphocytic leukemia and received induction chemotherapy, which was complicated by neutropenia. The patient’s respiratory function worsened and the patient died.

Molecular typing of carbapenem-resistant K. pneumoniae

From patient 1, the carbapenem-susceptible K. pneumoniae isolate (ARPG-318) was typed as ST147 carrying blactx-M-15, blaoxa-1, blashv-11, and blatem-1. PCR-based replicon typing did not detect a typeable plasmid. The lack of carbapenemase gene was confirmed by multiplex PCR. The carbapenem-resistant K. pneumoniae isolate ARPG-379 was identified as ST273. ARPG-379 had a positive modified Hodge test and carried blactx-M-15, blandm-7, blaoxa-1, blashv-11, and blatem-1. The presence of blandm-7 was confirmed by multiplex PCR and the product sequences match a previously reported blandm-7 (GenBank Accession No. JX262694.1). PCR-based replicon typing detected the IncA/C type plasmid.

From patient 2, the carbapenem-resistant K. pneumoniae isolate (ARP-664) was identified as ST656 and had a positive modified Hodge test. Carbapenemase gene sequences matched a previously reported blandm-1 (GenBank Accession No. FN396876.1) except for a C>T synonymous single-nucleotide polymorphism at position 564 (Table 1). PCR-based replicon typing did not detect a typeable plasmid. From patient 3, the carbapenem-resistant K. pneumoniae isolate (ARP-315) was identified as ST1 and carried blapha-1, blaoxa-1, and blashv-1. No carbapenemase genes were detected by either whole genome sequencing or multiplex PCR.

Discussion

To our knowledge, these are the first two cases of K. pneumoniae carrying blandm in the Philippines and the first report of the spread of ST273 and ST656. This report shows that the two K. pneumoniae carrying blandm are not epidemiologically linked; the isolates were genetically distinct. Each patient’s isolate had different multilocus sequence types and different blandm variants.

Previous surveillance of Enterobacteriaceae carrying genes encoding carbapenemases in the Philippines detected only blactm-26 from K. pneumoniae ST626 and ST903.13 K. pneumoniae ST273 was initially identified in Europe and has been reported in Italy, Norway, Russia, and the United Kingdom.14–17 These studies reported that ST273 isolates harbor various carbapenemase genes, including blakpc, blandm-1, and blandm, and recognized ST273 as having high epidemic potential. K. pneumoniae ST273 encodes a single allelic variant compared to ST147, which caused a nosocomial outbreak of NDM-1-producing clone in Mainland China.18,19 K. pneumoniae ST656 is a recently reported sequence type and has only been reported in China carrying blactx-M-14.20 Little is known about ST656, and the isolate from patient 2 (ARP-664) is the first report of ST656 carrying a carbapenemase gene. The isolate from patient 3 did not carry any known carbapenemase gene, but did carry a plasmidic AmpC beta-lactamase gene and ESBL-encoding genes, which are known to contribute to the carbapenem resistance phenotype when coexpressed with porin modification or loss.21

Ours is the fourth report of K. pneumoniae carrying blandm, which encodes the carbapenemase NDM-7, a newly described variant of NDM of particular concern because NDM-7 has a greater hydrolytic activity against carbapenems than NDM-1. The prior reports of K. pneumoniae carrying blandm were from two case reports of single patients and a report of an outbreak involving seven patients.22–24 There also have been six cases of infections with blandm-7 carrying E. coli.8,22,25–27 These isolates harboring blandm-7 have rapidly emerged in multiple continents since first being detected in 2012, and have been found in Germany, India, Japan, Spain, and the United States.8 This report contributes to the local and regional epidemiology of carbapenem-resistant Enterobacteriaceae. Tracking carbapenem-resistant Enterobacteriaceae and mechanisms of resistance are clinically significant because new antimicrobial agents, such as ceftazidime/avibactam, are not active against bacteria carrying metallo-beta-lactamase genes, such as blandm, but may be active against bacteria carrying blakpc. Surveillance will provide guidance on the utility of new antimicrobial agents to treat multidrug-resistant gram-negative infections.28 Given the concerns of its high epidemic potential, K. pneumoniae ST273 and blandm must be closely monitored and rapidly reported.

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Disclosure Statement

No competing financial interests exist.

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