Letter to the Editor

Emergence of Klebsiella pneumoniae producing dual carbapenemases (NDM-1 and OXA-232) and 16S rRNA methylase (armA) isolated from a Malaysian patient returning from India

Sir,

Carbapenemases are often used for the treatment of complicated infections caused by extended-spectrum β-lactamase-producing Klebsiella pneumoniae [1]. However, the emergence and spread of carbapenemases endanger the activity of these antibiotics as therapeutic options. To date, one case of carbapenemase (NDM-1)-producing K. pneumoniae has been reported in Malaysia [2]. High-level aminoglycoside resistance due to 16S RNA methylases has been increasingly documented, particularly in NDM-producing K. pneumoniae [1]. In this report, we describe the first imported case of multidrug-resistant (MDR) K. pneumoniae carrying dual carbapenemases (NDM-1 and OXA-232) and 16S rRNA methylase (armA) in Malaysia. The isolate was recovered from a urine specimen of an 80-year-old Malaysian male patient with benign prostatic hyperplasia. In May 2012 the patient developed urinary retention during a trip to India, where he was hospitalised for continuous urinary drainage using an indwelling catheter. Upon returning to Malaysia he was admitted to a local hospital in Kuala Lumpur for catheter removal. Two urine samples were taken for bacterial culture, one from the urinary catheter on admission to the hospital and another after the catheter was removed. Both urine samples yielded a pure growth of K. pneumoniae at a count of >10^6 bacilli/mL. These isolates formed mucoid, lactose-fermenting colonies on MacConkey agar and showed in vitro resistance to multiple antibiotics including imipenem. The patient was diagnosed as a case of urinary tract infection. He was treated with intravenous colistin and was discharged several days later.

Minimum inhibitory concentrations (MICs) of the isolate (designated as strain NDM-2012) were determined using Etest strips (bioMérieux, Marcy-l’Étoile, France). The isolate demonstrated resistance to all antibiotics tested including carbapenemases, but was susceptible to colistin and tigecycline. Isolate MICs are shown in Table 1. High levels of resistance to amikacin and gentamicin (MICs ≥ 256 μg/mL) were noted. The modified Hodge test showed a positive result for carbapenemase production.

PCR and sequence analyses were used to identify various resistance genes against β-lactam antibiotics, carbapenemases, fluoroquinolones, aminoglycosides and trimethoprim/sulfamethoxazole (SXT). All of the primers used in the detection of antibiotic resistance genes are listed in Supplementary Table S1. Table 1 gives the MICs for various antibiotics and the resistance genes detected in strain NDM-2012.

NDM, a prevalent carbapenemase in the Indian subcontinent, is now spreading in many parts of the world [1]. The NDM-1 gene was reported in 2013 from a Malaysian K. pneumoniae isolated from a urine specimen of a leukaemia patient without any history of travel to the Indian subcontinent [2]. OXA-48-like genes have never been reported previously from K. pneumoniae in Malaysia; however, several OXA-48-like variants (including OXA-48, OXA-181 and OXA-232) have been documented recently in the Asia-Pacific region [3].

This study describes the first imported case of a MDR K. pneumoniae carrying two carbapenemases (NDM-1 and OXA-232) in Malaysia. The co-existence of these two carbapenemases has been reported recently in several K. pneumoniae isolates, including one isolate from an American patient who was hospitalised in India [4], one isolate from Singapore [5] and two isolates from France [6].

Besides NDM-1 and OXA-232 carbapenemase genes, strain NDM-2012 had multiple resistance determinants, including β-lactamases (CTX-M-15, SHV-28 and OXA-1-like), aminoglycoside resistance genes (armA and aac(6’)-Ia-cr), fluoroquinolone resistance determinants (gyrA and parC mutations, qnrB and qnrS) and SXT resistance genes (sul1 and dfrA). Expression of multiple resistance determinants by strain NDM-2012 may explain the high-level resistance to multiple antibiotics from various classes (β-lactams including carbapenemases, β-lactam/β-lactamase inhibitors, aminoglycosides, fluoroquinolones and SXT), which is a typical feature of NDM-1–producing K. pneumoniae isolates [1].

High-level resistance to multiple aminoglycosides has become common in K. pneumoniae owing to the spread of 16S rRNA methylases, which have been increasingly reported in MDR Gram-negative bacteria worldwide, especially NDM-producing K. pneumoniae [1]. In this study, the armA gene was reported for the first time in Malaysia and was responsible for high-level resistance to both gentamicin and amikacin.

Multilocus sequence typing of the isolate was performed using the protocol available from http://www.pasteur.fr/mlst. Strain

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/mL)</th>
<th>Antibiotic resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>≥32</td>
<td>NDM-1 and OXA-232</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥32</td>
<td>β-Lactamase resistance genes</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥256</td>
<td>(CTX-M-15, SHV-28 and OXA-1)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥256</td>
<td>aacC2, armA</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥128</td>
<td></td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>≥256</td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥256</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥256</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥32</td>
<td>qnrB, aac(6’)-Ib-cr, mutations in gyrA (both Ser83 and Asp87 codons) and parC (Ser80 codon) sul1, dfrA</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>≥32</td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td>0.125</td>
<td>−</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1.5</td>
<td>−</td>
</tr>
</tbody>
</table>

Table 1

Minimum inhibitory concentrations (MICs) for various antibiotics and resistance-associated genes detected in strain NDM-2012.

http://dx.doi.org/10.1016/j.ijantimicag.2014.12.013

Please cite this article in press as: Al-Marzooq F, et al. Emergence of Klebsiella pneumoniae producing dual carbapenemases (NDM-1 and OXA-232) and 16S rRNA methylase (armA) isolated from a Malaysian patient returning from India. Int J Antimicrob Agents (2015).

http://dx.doi.org/10.1016/j.ijantimicag.2014.12.013
NDM-2012 belonged to sequence type 14 (ST14), in contrast to the first NDM-1-producing *K. pneumoniae* isolate from Malaysia that belonged to ST17 [2]. In fact, ST14 has been described as the most common sequence type in *K. pneumoniae* isolates carrying NDM-1 since its first description in 2009 and has been identified as the sequence type of *K. pneumoniae* isolates co-producing NDM-1 and OXA-232 in France [5] and the USA [4].

International travel has facilitated the spread of new antibiotic resistance determinants in many regions of the world. The emergence and dissemination of dual carbapenemase-producing *K. pneumoniae* with multiple resistance genes in several parts of the world (USA, Europe and Southeast Asia including Malaysia and Singapore) poses a therapeutic challenge to clinicians due to limited treatment choices. Implementation of stricter infection control measures is necessary to limit the spread of MDR bacteria in healthcare settings.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.12.013.

**Funding**

This study was supported by a University of Malaya postgraduate grant [PV037-2012A] and HIR-MOHE E000013-20001 (subprogramme 4) grant.

**Competing interests**

None declared.

**Ethical approval**

Not required.

**References**


Farah Al-Marzooq
Yun Fong Ngeow
Sun Tee Tay*

Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

*Corresponding author. Tel.: +60 3 7967 6661; fax: +60 3 7958 4844.
E-mail address: tayst@um.edu.my (S.T. Tay)

Received 23 December 2014
Accepted 31 December 2014