Emergence of clinical *Klebsiella pneumoniae* producing OXA-232 carbapenemase in Singapore

J. W. P. Teo[1], A. Kurup[2], R. T. P. Lin[3] and K. T. Hsien[4]

1) *Department of Laboratory Medicine, National University Hospital, 5 Lower Kent Ridge Road,* 2) *Duke-Nus Graduate Medical School, 8 College Road,* 3) *National Public Health Laboratory, Ministry of Health and* 4) *Pathology Department, Singapore General Hospital, 20 College Road, Academia, Level 7, Diagnostics Tower, Singapore*

Abstract

We report the emergence of OXA-232, a newly described OXA-48-like carbapenemase variant, in Southeast Asia. Molecular characterization of eight *Klebsiella pneumoniae* obtained from local and foreign patients reveals clonality of the isolates. $bla_{OXA-232}$ was located on a non-conjugative plasmid of 6141 base pairs (GenBank accession number JX423831.1).

Keywords: Antibiotic resistance, beta-lactam, infection
Original Submission: 28 May 2013; **Revised Submission:** 28 May 2013; **Accepted:** 12 June 2013
Article published online: 18 September 2013
New Microbe New Infect 2013; **1:** 13–15

Corresponding author: J. W. P. Teo, Department of Laboratory Medicine, 5 Lower Kent Ridge Road, Singapore 119074.
E-mail: Jeanette_teo@nuhs.edu.sg

The emergence and dissemination of carbapenemase-producing Enterobacteriaceae is an escalating global threat. As part of national surveillance efforts performed for carbapenem non-susceptible clinical Enterobacteriaceae isolates, we detected the presence of a new OXA-48-like variant, OXA-232. The novel OXA-232 was only very recently isolated in France from Enterobacteriaceae of patients who had travelled to India [1]. To the best of our knowledge, this is the first report providing molecular characterization of eight isolates carrying $bla_{OXA-232}$ in Southeast Asia.

The *Klebsiella pneumoniae* isolates were detected over a 2-month period from March to April 2013, with seven of the eight isolates originating from one hospital (Table 1). Bacterial isolates had their species identification confirmed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics GmHB, Bremen, Germany). No synergy was displayed with meropenem-dipicolinic acid, meropenem-boronic acid or meropenem-cloxacillin discs (Rosco Diagnostica A/S, Taastrup, Denmark), suggesting the absence of metallo-β-lactamases or class A β-lactamases. Antimicrobial susceptibility testing was performed with a VITEK-2 instrument and carbapenem MICs were confirmed with Etest (Biomérieux, Marcy L'Etoile, France) with susceptibility defined to European Committee on Antimicrobial Susceptibility Testing breakpoints. The isolates exhibited resistance to carbapenems, with imipenem MICs in the range of 2–8 mg/L and meropenem MICs $\geq$32 mg/L. The isolates were resistant to amikacin and gentamicin (MICs >256 mg/mL) as well as to levofloxacin (MIC >6 mg/mL). The isolates were sensitive to tigecycline and colistin (MICs <1.0 mg/mL).

Screening using PCR for a panel of β-lactamase genes was performed. This included genes for serine carbapenemases (KPC-type) [2], metallo-β-lactamases (NDM-type, VIM-type, IMP-type) [3], OXA-type carbapenemases [4] as well as extended spectrum β-lactamases (TEM-type, SHV-type, CTX-M-type, OXA-1, OXA-4, OXA-7, OXA-9) [3].

The PCR screening indicated that isolates were positive for bla_{OXA-48}. Full gene sequencing of OXA-48-type amplicons revealed 100% identity with $bla_{OXA-232}$ (GenBank accession number JX423831.1). OXA-232 differs from OXA-181 and OXA-48 by one and five amino acid substitutions, respectively [1]. The isolates were negative for KPC, metallo-β-lactamases and OXA-type extended spectrum β-lactamases. However, carriage of SHV and CTX-M extended spectrum β-lactamase genes was observed (Table 1).

Clonal relatedness of the isolates was investigated using pulsed-field gel electrophoresis (PFGE) of *K. pneumoniae* genomic DNA digested with the restriction enzyme *Spe*I. Visual inspection of the PFGE profiles indicated that all eight isolates belonged to a single pattern type and were therefore considered to be clonal [5].

Multilocus sequence typing (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) was performed on a representative isolate, which was determined to belong to sequence type (ST) 231 (Table 1). *Klebsiella pneumoniae* of ST231 have been identified in international studies as hosts of carbapenemases, in particular NDM-1 [6,7]. The $bla_{OXA-232}$ previously reported was found in *K. pneumoniae* belonging to ST14 [1]. Conjugation experiments were performed to assess the transferability of $bla_{OXA-232}$ from clinical *K. pneumoniae* isolates to drug susceptible, azide-resistant recipient *Escherichia coli* J53. Transconjugants were selected on Luria–Bertani agar containing sodium azide (100 mg/L) and

TABLE 1. Characteristics of *Klebsiella pneumoniae* clinical isolate producing OXA-232

Isolate	Country of isolation	Country of origin	Hospital	Specimen	Date of isolation	ST	β-lactamases	Plasmids Size (approximate in kb)	Replicon*
KP1	Singapore	Indonesia	Hospital X	Groin	19 March 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP2	Singapore	Indonesia	Hospital X	Abdominal fluid	22 March 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP3	Singapore	Singapore	Hospital X	Surgical wound	24 March 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP4	Singapore	USA, based in Indonesia	Hospital X	Cerebrospinal fluid	27 March 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP5	Singapore	Bangladesh	Hospital X	Urine	7 April 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP6	Singapore	Singapore	Hospital X	Sputum	9 April 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP7	Singapore	Singapore	Hospital X	Rectal swab	11 April 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like
KP8	Singapore	Singapore	Hospital Y	Pleural fluid	30 March 2013	231	OXA-232, TEM-1, SHV-12, CTX-M-15	4, 5, 6.1, 9, 70, 145	FIA, ColE-like

ST, Strain type as determined by multilocus sequence typing; —, information not available.
*Based on PCR-based replicon typing [9] with the inclusion of primers for ColE-type replicon [1].

meropenem (1 mg/L); however, no transconjugants were obtained, indicating that $bla_{OXA-232}$ was not transmissible.

The plasmid content of the *K. pneumoniae* isolates was analysed. All the isolates had similar plasmidic profiles, bearing plasmids of varying sizes between 4 and 145 kilobases (kb) (Table 1). Extraction of smaller plasmids using the QIAprep spin miniprep kits (Qiagen, Hilden, Germany) followed by S1 nuclease digestion revealed the presence of four small plasmids of 4–9 kb (Table 1). S1 nuclease digestion of whole genomic DNA followed by PFGE (S1-PFGE) [8] revealed the presence of two large plasmids of ~70 and 145 kb.

For the identification of plasmid incompatibility groups in the OXA-232 producers, PCR-based replicon typing based on the protocol by Johnson and Nolan [9] was used. Additionally, primers (RepCol181-For, 5′-TACGGATTGCGGATGTTGC C-3′; and RepCol181-Rev, 5′-GTGCTGGTGCGTCCATTTG G-3′) specific for the ColE-type replicon of OXA-181-bearing plasmid (GenBank accession number JN205800.1) were included [1,10]. Only ColE-type and FIA replicons were detected (Table 1). Southern hybridization analysis was performed on the S1 nuclease digested miniprep plasmid DNA, using a 641-base-pair digoxigenin-labelled probe amplified from the ColE-type replicon region using primers RepCol181-For and RepCol181-Rev. The probe hybridized to the 6-kb plasmid. The data collectively suggested that the 6-kb plasmid with ColE-type replicon could be similar to the 6.1-kb $bla_{OXA-232}$-bearing plasmid [1]. For sequence determination of the plasmid bearing $bla_{OXA-232}$, an inverse PCR and primer walking approach was employed. $bla_{OXA-232}$ carries a unique *Spe*I site, which facilitated the use of inverse PCR. Briefly, plasmid miniprep extractions of the *K. pneumoniae* isolates were subject to *Spe*I digestion then cleaned-up using a QIAquick PCR purification kit (Qiagen). Then 2 μL of this preparation were subjected to PCR using primers INV OXA1 (5′-CGATAAGGCTAATACACGCAT-3′) and INV OXA2 (5′-CAGGAGAAAATTATTCCCTAG-3′) that target the extremities of $bla_{OXA-232}$, allowing the sequences flanking $bla_{OXA-232}$ to be amplified. An approximately 6-kb DNA fragment was obtained by PCR and the entire sequence was determined by primer walking. The sequences for the 6-kb plasmid were determined for all eight isolates. Sequence analysis revealed that the 6-kb plasmid in all the isolates was identical to the 6141-base-pair plasmid characterized by Potron *et al.* [1]. This plasmid was non-conjugative, which accounted for the lack of transmissibility of $bla_{OXA-232}$ in conjugation assays.

Here, we highlight the emergence and outbreak potential of *K. pneumoniae* carrying a novel OXA-48-like variant in our local hospital setting. The isolates were derived from both local and foreign patients. Molecular profiling of the *K. pneumoniae* isolates indicated that they were highly similar, if not identical. However, because of insufficient clinical information, we are currently unable to ascertain the exact mechanism of spread. It remains to be seen if OXA-232 producers will become established locally, where OXA-181 is currently the most prevalent OXA-48 type carbapenemase, making up 25% of all carbapenemase producers in our surveillance studies [4].

References

1. Potron A, Rondinaud E, Poirel L *et al.* Genetic and biochemical characterisation of OXA-232, a carbapenem-hydrolysing class D β-lactamase from Enterobacteriaceae. *Int J Antimicrob Agents* 2013; 41: 325–329.
2. Balm MND, Ngan G, Jureen R, Lin RTP, Teo J. Molecular characterization of newly emerged bla_{KPC-2}-producing *Klebsiella pneumoniae* in Singapore. *J Clin Microbiol* 2012; 50: 475–476.
3. Teo J, Ngan G, Balm M, Jureen R, Krishnan P, Lin R. Molecular characterization of NDM-1 producing Enterobacteriaceae isolates in Singapore hospitals. *Western Pac Surveill Response J* 2012; 3: 19–24.
4. Balm MN, Ngan G, Jureen R, Lin RT, Teo JW. OXA-181-producing *Klebsiella pneumoniae* establishing in Singapore. *BMC Infect Dis* 2013; 13: 58–62.

5. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
6. Giske CG, Fröding I, Hasan CM *et al.* Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of blaNDM-1 in India, Sweden, and the United Kingdom. *Antimicrob Agents Chemother* 2012; 56: 2735–2738.
7. Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JD. Surveillance and molecular epidemiology of *Klebsiella pneumoniae* isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. *Antimicrob Agents Chemother* 2013; 57: 130–136.
8. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem* 1995; 226: 235–240.
9. Johnson TJ, Nolan LK. Plasmid replicon typing. *Methods Mol Biol* 2009; 551: 27–35.
10. Potron A, Nordmann P, Lafeuille E, Al Maskari Z, Al Rashdi F, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D β-lactamase from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2011; 55: 4896–4899.