Clonal Dissemination of OXA-232 Carbapenemase-Producing *Klebsiella pneumoniae* in Neonates

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ABSTRACT Five OXA-232 carbapenemase-producing *Klebsiella pneumoniae* isolates, belonging to the pandemic clone sequence type 15 (ST15), were isolated from neonates and coproduced *bla*_{CTX-M-15} and *bla*_{SHV-1} genes. All isolates were resistant to ertapenem (MICs of >32 µg/ml) and meropenem (MICs of 4 to 8 µg/ml) and susceptible or intermediate to imipenem (MICs of 1 to 2 µg/ml). The *bla*_{OXA-232} gene was located on a ColE-type transformable plasmid of 6,141 bp. To the best of our knowledge, this is the first report of OXA-232 carbapenemase among clinical isolates in China.

KEYWORDS *Klebsiella pneumoniae*, OXA-232, ST15, clonal dissemination, neonates

Carbapenemase-producing *Enterobacteriaceae* isolates are usually extensively drug resistant, and infections caused by these pathogens present a serious clinical challenge, especially for pediatric patients (1). Based on the data of the CHINET antimicrobial resistance surveillance program for 2005 to 2014, the rates of *Klebsiella pneumoniae* resistance to imipenem and meropenem increased markedly, from 1.3% to 14.6% and from 0% to 15.2%, respectively (2). Production of *Klebsiella pneumoniae* carbapenemase (KPC), NDM-1-type metallo-β-lactamase, and oxacillinase is the main mechanism underlying resistance to carbapenems. The OXA-48 carbapenemase, which characteristically is highly resistant to ertapenem, was initially identified in 2001 in *K. pneumoniae* isolates from Istanbul, Turkey; since then, many variants of *bla*_{OXA-48} have been described, including *bla*_{OXA-162} from Turkey, *bla*_{OXA-163} from Argentina, *bla*_{OXA-181} from India, *bla*_{OXA-204} from Tunisia, and *bla*_{OXA-232} from France and Singapore (3–9). Here we report a small clonal dissemination of OXA-232 carbapenemase-producing *K. pneumoniae* clinical isolates, representing the first detection in China of *Enterobacteriaceae* clinical strains bearing *bla*_{OXA-232}.

Five nonduplicate clinical strains of *K. pneumoniae* were isolated in April to June 2016 from five patients in the neonatal intensive care unit (NICU) of a university children's hospital in Shanghai, China. The presence of β-lactamase genes was confirmed by specific PCR and sequencing, as described previously (10–12). All *K. pneumoniae* isolates were positive for *bla*_{OXA-232}, *bla*_{SHV-1}, and *bla*_{CTX-M-15}, belonging to a single pulsed-field gel electrophoresis (PFGE) type identified as sequence type 15 (ST15) by multilocus sequence typing (MLST) (Fig. 1A). The outer membrane porins (OMPs) were analyzed by SDS-PAGE (13), which revealed the presence of two main OMPs, OmpK35 and OmpK36 (Fig. 1B), in the five stable ertapenem-resistant isolates.

Transformants harboring the plasmid with *bla*_{OXA-232} were selected on Mueller-Hinton agar containing 50 µg/ml ampicillin, with *bla*_{OXA-232} determination by PCR and DNA sequencing. All positive transformants were negative for *bla*_{SHV-1} and *bla*_{CTX-M-15}.

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FIG 1 (A) DNA fingerprinting of five OXA-232 carbapenemase-producing *K. pneumoniae* strains by PFGE. (B) OMP analysis of five OXA-232 carbapenemase-producing *K. pneumoniae* strains by SDS-PAGE.

Plasmid DNA sequencing localized *bla*_{OXA-232} on a small plasmid of 6,141 bp, belonging to the ColE type. This targeted plasmid contained nine open reading frames, i.e., MobA, MobB, MobD, Δ MobC, Δ ISEcp1, *bla*_{OXA-232}, Δ LysR, Δ EreA, and RepA (Fig. 2). The results of antimicrobial susceptibility testing indicated that all clinical isolates were susceptible or intermediate to imipenem and susceptible to tigecycline and colistin but resistant to other antimicrobial agents. The ertapenem MICs of transformants increased at least 8-fold, compared with the recipients (Table 1).

Differing from *bla*_{OXA-181} by one amino acid substitution, *bla*_{OXA-232} was initially isolated from *Enterobacteriaceae* collected from patients in France who had traveled previously to India and then was sporadically detected in *Klebsiella pneumoniae* in Singapore, the United States, and South Korea (9, 14, 15). To some extent, the first description of *bla*_{OXA-232} in *K. pneumoniae* clinical isolates highlights the potential risk of spreading in pediatric hospitals and the importance of carbapenem-resistant *Enterobacteriaceae* (CRE) surveillance indicated by ertapenem resistance.

Previously reported *K. pneumoniae* with *bla*_{OXA-232} belonged to ST14 and ST231 and *Escherichia coli* belonged to ST2968 (3, 9), while the dominant epidemic ST in our study was ST15. As a successful international clone, ST15 (as well as ST14 and ST231) *K. pneumoniae* has been identified in various countries in association with a variety of β -lactamases, mainly including NDM-1, CTX-M-15, KPC-2, and OXA-48-like (16, 17). In our study, *bla*_{CTX-M-15} results were negative among transformants but positive in donor strains, demonstrating that *bla*_{CTX-M-15} and *bla*_{OXA-232} were located in different plasmids. Compared with two *K. pneumoniae* isolates (RAN and DEL) detected in France (3), the *K. pneumoniae* isolates in our study showed similar drug resistance profiles, with ertapenem MIC values of $>32 \mu\text{g/ml}$, and both remained susceptible to colistin and

FIG 2 Overview of the target plasmid carrying *bla*_{OXA-232}, from five OXA-232 carbapenemase-producing *K. pneumoniae* strains. MobC, transcription regulator Δ LysR, and erythromycin esterase Δ EreA, partial coding sequences; MobA, MobB, MobD, RepA, insertion sequence Δ ISEcp1, and *bla*_{OXA-232}, complete coding sequences.

TABLE 1 *In vitro* activities of antimicrobial agents against *K. pneumoniae* isolates and their transformants

Strain	β -Lactamase(s)	MIC (mg/liter) ^a															
		ETP	IPM	MEM	CSL	CZO	CXM	CTX	CAZ	CMZ	TGC	FEP	PIP	TZP	CIP	LEV	GEN
KP1	OXA-232, SHV-1/ CTX-M-15	32	1	4	128/64	>32	>64	>32	>32	32	1	>32	>256	>256/4	>8	>16	>32
KP1-T	OXA-232	0.25	0.5	0.06	8/4	8	16	≤0.25	≤0.25	32	≤0.25	≤0.5	256	64/4	≤0.25	≤0.5	≤2
KP2	OXA-232, SHV-1/ CTX-M-15	32	2	8	128/64	>32	>64	>32	>32	16	1	>32	>256	>256/4	>8	>16	>32
KP2-T	OXA-232	0.5	0.5	0.06	16/8	8	8	≤0.25	≤0.25	8	≤0.25	≤0.5	128	32/4	≤0.25	≤0.5	≤2
KP3	OXA-232, SHV-1/ CTX-M-15	64	1	4	>128/64	>32	>64	>32	>32	32	2	>32	>256	>256/4	>8	>16	>32
KP3-T	OXA-232	0.25	0.5	0.06	16/8	8	8	≤0.25	≤0.25	≤0.5	≤0.25	≤0.5	256	64/4	≤0.25	≤0.5	≤2
KP4	OXA-232, SHV-1/ CTX-M-15	32	1	4	>128/64	>32	>64	>32	>32	≤0.5	1	>32	>256	>256/4	>8	>16	>32
KP4-T	OXA-232	0.25	0.5	≤0.03	16/8	8	8	≤0.25	≤0.25	≤0.5	≤0.25	≤0.5	>256	64/4	≤0.25	≤0.5	≤2
KP5	OXA-232, SHV-1/ CTX-M-15	64	2	4	128/64	>32	>64	>32	>32	≤0.5	1	>32	>256	>256/4	>8	>16	>32
KP5-T	OXA-232	0.25	0.5	0.06	16/8	8	8	≤0.25	≤0.25	1	≤0.25	≤0.5	128	64/4	≤0.25	≤0.5	≤2
<i>E. coli</i> DH5 α		≤0.03	0.125	≤0.03													

^aETP, ertapenem; IPM, imipenem; MEM, meropenem; CSL, cefoperazone-sulbactam; CZO, ceftazidime; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; CMZ, cefmetazole; TGC, tigecycline; FEP, cefepime; PIP, piperacillin; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; LEV, levofloxacin; GEN, gentamicin; AMK, amikacin; CST, collistin; ATM, aztreonam; CHL, chloramphenicol; MNO, minocycline; SXT, trimethoprim-sulfamethoxazole.

TABLE 2 Clinical features of patients infected or colonized with OXA-232 carbapenemase-producing *K. pneumoniae* isolates

Patient no.	Age	Sex	Underlying condition	Days of hospital stay before infection/total days	Specimen	Invasive procedure ^a	Prior antibiotic exposure	Antimicrobial therapy	Infection or colonization	Status at hospital discharge
1	4 mo	Male	Neonatal pulmonary hypoplasia, neonatal pneumonia, septicemia	1/180	Sputum	Endotracheal intubation	SAM, MEM, VRO, CRO, IPM	SAM, MEM, FOS, SCF, VRO, CAZ, AMK, CIP	Infection	Died
2	35 d	Male	Congenital laryngeal cartilage dysplasia, malrotation	26/46	Sputum	Endotracheal intubation, PICC	CDZ, CPC, SAM, MEM	FOS, MEM, SCF	Infection	Recovered
3	4 d	Male	Neonatal respiratory distress syndrome, persistent pulmonary hypertension of newborn, congenital heart disease	4/14	Sputum	Endotracheal intubation	SAM, CTX	SAM, MEM	Infection	Recovered
4	16 d	Male	Hyperbilirubinemia, hemolytic disease of newborn	14/23	Urine	None	SAM	SAM	Colonization	Recovered
5	4 mo	Male	Neonatal pulmonary hypoplasia, anemia, neonatal abdominal distension	98/114	Urine	UVC, endotracheal intubation, intestinal surgery	SAM, CPC	MEM, FOS	Infection	Recovered

^aPICC, peripherally inserted central catheter; UVC, umbilical venous catheter; AMK, amikacin; CDZ, cefodizime; CPC, cefepime; CIP, ciprofloxacin; CTX, cefotaxime; CAZ, ceftazidime; SAM, ampicillin-sulbactam; MEM, meropenem; IPM, imipenem; VRO, vancomycin; CRO, ceftriaxone; FOS, fosfomycin; SCF, cefoperazone-sulbactam.

tigecycline. However, *K. pneumoniae* RAN was also resistant to imipenem and meropenem, with MIC values of $>32 \mu\text{g/ml}$, which differed from the findings in our study (MIC values of 1 to $2 \mu\text{g/ml}$ for imipenem and 4 to $8 \mu\text{g/ml}$ for meropenem), despite identical structures of the ColE-type plasmid. To date, there have been limited data regarding the clinical features, treatment, and prognosis of patients infected with isolates with *bla*_{OXA-232}. In this study, all patients were treated with meropenem plus other antimicrobial agents, and four recovered, although one died (Table 2). According to the report by Tzouveleakis et al. (18), the therapeutic efficacy of carbapenems for patients infected with carbapenemase-positive *K. pneumoniae* would increase from 66.7% with a MIC of $8 \mu\text{g/ml}$ to 71.4% with a MIC of $4 \mu\text{g/ml}$, indicating that carbapenems could provide some therapeutic benefit in infections caused by strains like ours.

Additionally, it was demonstrated that a novel OmpK36 variant was associated with ertapenem resistance, reduced susceptibility to meropenem, and susceptibility to imipenem (13). In this study, sequencing of the *ompK35* and *ompK36* genes from five strains showed less than 80% and 90% amino acid identities with other *K. pneumoniae* OmpK35 and OmpK36 proteins (GenBank accession numbers [KX528035.1](#), [GU945359.1](#), [GQ485678.1](#), [HM000077.1](#), and [KY019192.1](#)), respectively. The differences in amino acid sequences may reveal OmpK35 and OmpK36 protein variants in these strains and may explain the carbapenem susceptibility test results, similar to the previous description.

Here, we highlight the emergence and outbreak potential of *K. pneumoniae* carrying a novel OXA-48-like variant in a health care setting. Although there is limited evidence for the clonal dissemination of *bla*_{OXA-232}, we speculate that low levels of hand hygiene compliance and failure of contact precautions might have contributed to the infection or colonization of five patients during hospitalization. Active surveillance for CRE should be performed to restrict the spread of these multidrug-resistant clinical isolates, especially for pediatric patients.

Accession number(s). The complete nucleotide sequence of the plasmid carrying *bla*_{OXA-232} is available in GenBank under accession number [KY454616](#).

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