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SHORT REPORT

First Report of OXA-181-Producing *Klebsiella pneumoniae* in China

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Abstract: We present here the first report of an OXA-181-producing *Klebsiella pneumoniae* isolated from the fecal specimen of a patient in China. The OXA-181-encoding gene $bla_{\text{OXA-181}}$ was located on a 51 kb IncX3-type plasmid. Conjugation assay and whole-genome sequencing analysis revealed that this transferrable plasmid in the *K. pneumoniae* isolate might have originated from *Escherichia coli* and have the potential to mediate the spread of $bla_{\text{OXA-181}}$.

Keywords: OXA-181, *Klebsiella pneumoniae*, IncX3 plasmid, China, human

Introduction

In 2001, a new carbapenem-hydrolyzing class D β-lactamase named OXA-48 was first identified in *Klebsiella pneumoniae* in Turkey.[1] Since this report, several variants of OXA-48 (including OXA-162, OXA-204, OXA-232, OXA-245, and OXA-181 et. al) have been identified in *Enterobacteriaceae* worldwide.[2,3] Of these variants, OXA-181, which contains four amino acid substitutions, was first reported in India in 2007[4] and has since been identified, mainly in *K. pneumoniae* and *E. coli*, in several countries (UK, USA, and Denmark), showing a trend of increasing prevalence in in *Enterobacteriaceae*.[5,6] The gene encoding OXA-181, $bla_{\text{OXA-181}}$, is often found to be located on plasmids of incompatibility group (*Inc*) X that are defined as X3 type (*IncX3*).[7–9] These plasmids are known to disseminate various carbapenemase genes, including bla_{KPC} and bla_{NDM}.[10,11] To date, only two OXA-181-producing *E. coli* isolates have been reported in China, namely, in Sichuan and Henan.[12,13] Here, we present the first report of the identification of a *K. pneumoniae* isolate harboring $bla_{\text{OXA-181}}$ in China.

Materials and Methods

Clinical Isolate

A 60-year-old male patient was admitted to the general practice inpatient department of the Jinhua Municipal Central Hospital in Zhejiang Province, China, for 11 days in May 2019 due to headache and pain in the right finger with unknown causes. Clinical laboratory tests were conducted on the patient's blood, urine, and fecal samples. These tests revealed no abnormal results and no infection symptoms were observed. Neither sputum culture nor blood culture was conducted and no antibiotic treatment was provided before or during hospitalization. The patient was discharged after the pain was alleviated. However, during the discharge screening, a *K. pneumoniae* isolate carrying $bla_{\text{OXA-181}}$ was recovered from the fecal sample on the MacConkey agar

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medium supplemented with 0.3 mg/L meropenem; the isolate was designated *K. pneumoniae* 709. The strain was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using a spectrometer from Bruker, Germany.

Antimicrobial Susceptibility Testing, Identification of Antibiotic Resistance Genes, and Conjugation Assay

Antimicrobial susceptibility of strain 709 and its conjugant, designated J709 was determined by the micro broth dilution method and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The resistance breakpoints from the CLSI were used for imipenem, meropenem, ertapenem, ceftazidime, cefotaxime, piperacillin/tazobactam, cefoperazone/sulbactam, ceftazidime/avibactam, cefepime, ciprofloxacin, amikacin, and aztreonam. The resistance breakpoints for polymyxin E and tigecycline were interpreted according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (available at http://www.eucast.org/clinical_breakpoints/). Polymerase chain reaction (PCR) was used to detect $bla_{OXA-181}$ using the following pair of primers: OXA48-F, 5′-TCAGTAGCT GAACAGGAGGGA-3′ and OXA48-R, 5′-TTCGAGCCG CAGACAATT-3′. To determine the transferability of $bla_{OXA-181}$, a conjugation assay was performed using *E. coli* EC600 as the recipient strain. The conjugants were selected on a MacConkey agar medium containing 600 mg/L rifampicin and 1 mg/L meropenem. MALDI-TOF MS and PCR with the same primers as above were used to confirm the presumptive conjugant. Besides, the Multilocus sequence typing (MLST) - was performed using SRST2.[14]

Whole-Genome Sequencing and Plasmid Analysis

Genomic DNA was extracted by using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA). Whole genome sequencing was conducted using the Illumina HiSeq X10 (San Diego, CA, USA) and Nanopore MinION (Oxford, UK) sequencer platforms. The draft genomes were assembled using SPAdes v3.13.1.[15] The complete plasmid sequence was annotated using RAST tool.[16] Complete plasmid sequence alignment was conducted using BLAST Ring Image Generator (BRIG).[17]

Results and Discussion

In the antimicrobial susceptibility testing, 709 and J709 showed a low level of hydrolytic activity against carbapenems (Table 1), consistent with another report.[18] Strains with low carbapenem MICs are often ignored and not referred for further investigations in clinical practice; therefore, $bla_{OXA-181}$ may have already spread in China despite there being only two reports from this country about strains harboring this gene.[12,13] In addition, the OXA-181-producing strains with high carbapenem MICs identified previously also carried additional resistance mechanisms, such as porin deficiency.[19,20] By contrast, isolate 709 was still susceptible to the other tested antimicrobial agents (cefotaxime, ceftazidime, aztreonam, amikacin, and ciprofloxacin).

To characterize the genetic environment of $bla_{OXA-181}$ and the molecular type of *K. pneumoniae* 709, whole-genome sequencing was performed. $bla_{OXA-181}$ has been detected in various *K. pneumoniae* strains (ST43, ST147, ST836, ST11, ST61, ST25, ST307, ST709, etc.)[5,20,21] worldwide. Multilocus sequence typing (MLST) analysis indicated that *K. pneumoniae* 709 belonged to a novel sequence typing with one allele differing from ST432. Further analysis indicated that $bla_{OXA-181}$, together with the quinolone resistance gene *qnrS1*, was located on a 51 kb IncX3-type plasmid, designated by pKP709-OXA-181. The complete sequence of this plasmid has been deposited in GenBank under accession number MN227183. The conjugation assay revealed that pKP709-OXA-181 was a conjugative plasmid and BLAST analysis showed that the plasmid was identical to the *E. coli* plasmids pEC21-OXA-181 (GenBank accession number

Table 1 Susceptibilities of the *K. pneumoniae* 709, Conjugant J709 and Recipient EC600

Strains	MIC (mg/L)													
	IPM	MEM	ETP	CAZ	CTX	TZP	SCF	CAV	FEP	PE	TGC	CIP	AK	ATM
709	8	4	8	≤2	≤4	32/4	≤8/4	≤0.5/4	≤4	≤0.5	≤0.25	≤1	≤4	≤4
J709	4	2	4	≤2	≤4	≤8/4	≤8/4	≤0.5/4	≤4	≤0.5	≤0.25	2	≤4	≤4
EC600	≤1	≤1	≤2	≤2	≤4	≤8/4	≤8/4	≤0.5/4	≤4	≤0.5	≤0.25	≤1	≤4	≤4

Abbreviations: IMP, imipenem; MEM, meropenem; ETP, ertapenem; CAZ, Ceftazidime; CTX, Cefotaxime; TZP, Piperacillin/Tazobactam; SCF, Cefoperazone/Sulbactam; CAV, ceftazidime/avibactam; FEP, Cefepime; PE, polymyxin E; TGC, tigecycline; CIP, ciprofloxacin; AK, amikacin; ATM, aztreonam.

MG893567)[13] and pOXA181_EC14828 (GenBank accession number KP400525)[12] (100% coverage and 99% sequence similarity) reported from China (Figure S1). This finding suggested that our *K. pneumoniae* plasmid pKP709 might have been derived from *E. coli*. Furthermore, pKP709-OXA-181 also harbored IS*Ecp1*, an efficient genetic vehicle for disseminating clinically significant extended-spectrum β-lactamases, upstream of $bla_{\text{OXA-181}}$, as previously reported.[13,22] In this isolate, the *qnrS1* gene, which was located between a truncated *IS2* insertion sequence and a Tn3-like transposon, did not confer resistance to fluoroquinolones (Table 1). The genetic context of the $bla_{\text{OXA-181}}$ gene was the same as that of pEC21-OXA-181 as described by Qin et al.[13] These findings suggest that close surveillance of resistance strains in the human gut flora should be included as a routine clinical practice to prevent occurrence of infections, especially among immunocompromised patients.

Conclusion

In summary, we present here the first report of an OXA-181-producing *K. pneumoniae* in China. The genetic environment of $bla_{\text{OXA-181}}$ is identical to a previously described *E. coli* plasmid, indicating that the *K. pneumoniae* strain might have acquired the gene from *E. coli* via the transferable IncX3-type plasmid. IncX3-type plasmids harboring $bla_{\text{OXA-181}}$ could become the main vehicle for the spread of $bla_{\text{OXA-181}}$ in future in China. Moreover, because the gastrointestinal tract is a major reservoir of antibiotic resistance genes, screening of fecal samples for $bla_{\text{OXA-181}}$ is recommended to prevent its possible rapid dissemination via the IncX3-type plasmid.

Ethics and Consent Statement

The conduction of this research and publication of case details were approved by the Ethics Committee of Jinhua Municipal Central Hospital (2019-135-001) and the written informed consent was acquired from the patient to have the case details and any accompanying images published.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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