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

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 blaOXA-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 blaOXA-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. 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. Of these variants, OXA-181, which contains four amino acid substitutions, was first reported in India in 2007 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 Enterobacteriaceae. The gene encoding OXA-181, blaOXA-181, is often found to be located on plasmids of incompatibility group (Inc) X that are defined as X3 type (IncX3). These plasmids are known to disseminate various carbapenemase genes, including blaKPC and blaNDM. To date, only two OXA-181-producing E. coli isolates have been reported in China, namely, in Sichuan and Henan. Here, we present the first report of the identification of a K. pneumoniae isolate harboring blaOXA-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 blaOXA-181 was recovered from the fecal sample on the MacConkey agar
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, ceferazone/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 GAAACAGAGGA-3' and OXA48-R, 5'-TTGAGGCG CAGACAAATT-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.

**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 Illumina HiSeq X10 (San Diego, CA, USA) and Nanopore MinION (Oxford, UK) sequencer platforms. The draft genomes were assembled using SPAdes v3.13.1. The complete plasmid sequence was annotated using RAST tool. Complete plasmid sequence alignment was conducted using BLAST Ring Image Generator (BRIG).

**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. 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. In addition, the OXA-181-producing strains with high carbapenem MICs identified previously also carried additional resistance mechanisms, such as porin deficiency. 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.) worldwide. Multilocus sequence typing (MLST) analysis indicated that K. pneumoniae 709 belonged to a novel sequence typing and the molecular type of K. pneumoniae 709, whole-genome sequencing was performed. bla\_(OXA-181) 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 conjugal plasmid and BLAST analysis showed that the plasmid was identical to the E. coli plasmids pEC21-OXA-181 (GenBank accession number

| 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  |

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

Abbreviations: IMP, imipenem; MEM, meropenem; ETP, ertapenem; CAZ, Ceftazidime; CTX, Cefotaxime; TZP, Piperacillin/Tazobactam; SCF, Ceferazone/Sulbactam; CAV, Ceftazidime/Avibactam; FEP, Cefepime; PE, polymyxin E; TGC, Tigecycline; CIP, Ciprofloxacin; AK, Amikacin; ATM, Aztreonam.
and pOXA181_EC14828 (GenBank accession number KP400525) (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 ISEcp1, an efficient genetic vehicle for disseminating clinically significant extended-spectrum β-lactamases, upstream of blaOXA-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 blaOXA-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 blaOXA-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 blaOXA-181 could become the main vehicle for the spread of blaOXA-181 in future in China. Moreover, because the gastrointestinal tract is a major reservoir of antibiotic resistance genes, screening of fecal samples for blaOXA-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.

References

1. Poirel L, Heritier C, Tolun V, et al. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother. 2004;48(1):15–22. doi:10.1128/AAC.48.1.15-22.2004
2. Lowe M, Kock MM, Coetzee J, et al. Klebsiella pneumoniae ST307 with blaOXA-181, South Africa, 2014–2016. Emerg Infect Dis. 2019;25:739–747. doi:10.3201/eid2504.181482
3. LJ R, Hu Jer AM, Rudin SD, et al; for the Antibacterial Resistance Leadership Group (ARLG). NDM-5 and OXA-181 beta-lactamases, a significant threat continues to spread in the Americas. Antimicrob Agents Chemother. 2017;61:e00454–17. doi:10.1128/AAC.00454-17
4. Castanheira M, Deshpande LM, Mathai D, et al. Early dissemination of NDM-1- and OXA-181-producing enterobacteriaceae in Indian hospitals: report from the SENTRY antimicrobial surveillance program, 2006–2007. Antimicrob Agents Chemother. 2011;55(3):1274–1278. doi:10.1128/AAC.01497-10
5. Findlay J, Hopkins KL, Loy R, et al. OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 to 2014. J Antimicrob Chemother. 2017;72:1340–1349. doi:10.1093/jac/dcx012
6. L R, Overballe-Petersen S, Hansen F, et al. Escherichia coli sequence type 410 is causing new international high-risk clones. mSphere. 2018;3:e00337–18. doi:10.1128/mSphere.00337-18
7. Uwaezuoke NS, Kieffer N, Iregbu KC, et al. First report of OXA-181 and NDM-1 from a clinical Klebsiella pneumoniae isolate from Nigeria. Int J Infect Dis. 2017;61:1–2. doi:10.1016/j.ijid.2017.05.004
8. Izdebski R, Baraniak A, Zabicka D, et al. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. J Antimicrob Chemother. 2018;73:620–625. doi:10.1093/jac/dcx457
9. Pulss S, Semmler T, Prenger-Berninghoff E, et al. First report of an Escherichia coli strain from swine carrying an OXA-181 carbapenemase and the colistin resistance determinant MCR-1. Int J Antimicrob Agents. 2017;50(2):232–236. doi:10.1016/j.ijantimicag.2017.03.014
10. Fortini D, Villa L, Feudi C, et al. Double copies of bla(KPC-3)::tn4401a on an IncX3 Plasmid in Klebsiella pneumoniae successful clone ST512 from Italy. Antimicrob Agents Chemother. 2016;60:646–649. doi:10.1128/AAC.01886-15
11. Ho P-L, Wang Y, Liu MC-J, et al. IncX3 epidemic plasmid carrying blaNDM-5 in escherichia coli from swine in multiple geographic areas in China. Antimicrob Agents Chemother. 2018;62:e02295–17. doi:10.1128/aac.02295-17
12. Liu Y, Feng Y, Wu W, et al. First report of OXA-181-producing escherichia coli in China and characterization of the isolate using whole-genome sequencing. Antimicrob Agents Chemother. 2015;59:5022–5025. doi:10.1128/AAC.00442-15
13. Qin S, Cheng J, Wang P, et al. Early emergence of OXA-181-producing escherichia coli ST410 in China. J Glob Antimicrob Resist. 2018;15:215–218. doi:10.1016/j.jgar.2018.06.017
14. Inouye M, Dashnow H, Raven LA, et al. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. Genome Med. 2014;6:90. doi:10.1186/s13073-014-0090-6
15. Bankevich A, Nurk S, Antipov D, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19:455–477. doi:10.1089/cmb.2012.0021
16. Overbeek R, Olson R, Puschn GD, et al. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res. 2014;42:D206–14. doi:10.1093/nar/gkt1226
17. Alikhan NF, Petty NM, Ben Zakour NL, et al. BLAST ring image generator (BRIG): simple prokaryote genome comparisons. BMC Genomics. 2011;12:402. doi:10.1186/1471-2164-12-402
18. Evans BA, Amyes SG. OXA beta-lactamases. Clin Microbiol Rev. 2014;27:241–263. doi:10.1128/CMR.00117-13
19. Oueslati S, Nordmann P, Poirel L. Heterogeneous hydrolytic features for OXA-48-like beta-lactamases. *J Antimicrob Chemother*. 2015;70:1059–1063. doi:10.1093/jac/dku524

20. S KY K, Shigemoto N, Kuwahara R, et al. Imipenem-susceptible, meropenem-resistant klebsiella pneumoniae producing OXA-181 in Japan. *Antimicrob Agents Chemother*. 2015;59:1379–1380. doi:10.1128/AAC.04330-14

21. Machuca J, Lopez-Cerero L, Fernandez-Cuenca F, et al. OXA-48-like-producing klebsiella pneumoniae in Southern Spain in 2014–2015. *Antimicrob Agents Chemother*. 2019;63:e01396-18. doi:10.1128/AAC.00779-19

22. Potron A, Nordmann P, Lafeuille E, et al. Characterization of OXA-181, a carbapenem-hydrolyzing class D-lactamase from Klebsiella pneumoniae. *Antimicrob Agents Chemother*. 2011;55:4896-4899. doi:10.1128/AAC.00481-11