



Co-occurrence of *fosA5*, *bla*_{SHV-145} and *bla*_{OXA-48} among a *Klebsiella pneumoniae* high-risk ST16 from a tertiary hospital in China: focusing on the phylogeny of OXA-48 genes from global *Klebsiella pneumoniae* isolates

Yanmei Sun¹ · Wei Chen² · Shiwei Wang¹ · Xiaoli Cao³

Received: 23 November 2020 / Accepted: 29 June 2021 / Published online: 17 August 2021
© Sociedade Brasileira de Microbiologia 2021

Since the OXA-48-type carbapenem-hydrolyzing class D β -lactamase was reported in a *Klebsiella pneumoniae* isolate from Istanbul (Turkey) in 2001 [1], this carbapenemase has been widely distributed among *Enterobacteriales*, with significant geographical differences [2, 3]. Albeit outbreak of nosocomial infections caused by OXA-48-producing *K. pneumoniae* has been frequently reported in the Mediterranean area and European countries [4–6]. The situation is less common in China, until the nosocomial outbreak of OXA-48-producing *K. pneumoniae* ST147 and ST383 was reported in a Chinese Hospital [7]. Subsequently, clonal dissemination of KPC-2- and OXA-48-coproducing *K. pneumoniae* sequence type 11 and the nosocomial outbreak of OXA-48-producing *K. pneumoniae* caused by clonal expansion of ST11-KL64 sublineages were found in Taiwan [8, 9].

In this study, we provided data on the genomic characterization of an imipenem intermediate *K. pneumoniae* strain isolated from the blood culture of a 56-year-old male patient who suffered from bacteremia and septic shock in May 2017. Antimicrobial susceptibility testing toward imipenem, meropenem, piperacillin, ticarcillin, cefoperazone, cefuroxime, cefazolin, ceftazidime, ampicillin, cefepime, ceftriaxone, cefepime, amikacin, gentamicin, chloramphenicol, levofloxacin, ciprofloxacin, and trimethoprim/sulfamethoxazole was performed by broth microdilution and that of fosfomycin was determined by the agar dilution method using Mueller Hinton medium supplemented with 25 μ g/mL glucose-6-phosphate, and the results were interpreted according to the CLSI 2020 (M100-30ED) guideline [10]. To characterize this strain in detail, whole genome sequencing was further performed using an Illumina MiniSeq plus Pacbio Sequencing. Based on the whole genome sequence, subsequent multi-locus sequence typing (MLST) was determined by uploading the genome to the webtool MLST v2.0 (<https://cge.cbs.dtu.dk/services/MLST/>), and antimicrobial resistance genes and plasmid replicons were identified by ResFinder 3.2 and PlasmidFinder 2.1 (<https://cge.cbs.dtu.dk/services/>). The plasmid carrying *bla*_{OXA-48} was detailedly characterized by circular plasmid map and comparison of plasmid structures. Additionally, the phylogenomic tree of *bla*_{OXA-48} from global *K. pneumoniae* isolates was further constructed to observe the evolutionary relationship of *bla*_{OXA-48}. Briefly, the nucleotide sequences of *bla*_{OXA-48} gene of 576 strains were compared by muscle, and then single nucleotide polymorphism (SNP) sites were used to extract SNPs from multiple alignment species. Finally, the maximum likelihood tree was constructed using RAxML software [11].

Yanmei Sun and Wei Chen equally contributed to this work

✉ Shiwei Wang
wangsw@nwu.edu.cn

✉ Xiaoli Cao
cao-xiao-li@163.com

¹ Key Laboratory of Resources Biology and Biotechnology in Western China, Ministry of Education, College of Life Science, Northwest University, Xi'an 710069, People's Republic of China

² Clinical Research Center, the Second Hospital of Nanjing, Nanjing University of Chinese Medicine, Nanjing 210003, People's Republic of China

³ Department of Laboratory Medicine, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Zhongshan Road, 321#, Gulou District, Nanjing, Jiangsu, People's Republic of China

Table 1 Antimicrobial susceptibility testing results for the OXA-48-producing *Klebsiella pneumoniae*

Antimicrobials	MIC ($\mu\text{g/mL}$) ^a	Inter-pretation ^b
Fosfomycin	> 256	R
Piperacillin	> 128	R
Ticarcillin	> 128	R
Cefoperazone	> 64	R
Cefuroxime	> 32	R
Cefoxitin	> 32	R
Ampicillin	> 32	R
Meropenem	> 16	R
Chloramphenicol	16	R
Levofloxacin	8	R
Cefazolin	> 8	R
Ciprofloxacin	4	R
Ceftazidime	4	S
Imipenem	2	I
Amikacin	< 4	S
Ceftriaxone	1	S
Gentamicin	< 1	S
Cefepime	< 2	S
Trimethoprim/sulfamethoxazole	< 0.5	S

^a An antimicrobial susceptibility testing was performed with standard broth microdilution method and interpreted based on the criteria from the Clinical and Laboratory Standards Institute guidelines (M100-30ED-2020)

^b R, resistant; S, susceptible; I, intermediate

Antimicrobial susceptibility testing showed that this strain was intermediate to imipenem (2 $\mu\text{g/mL}$). However, resistance to meropenem (8 $\mu\text{g/mL}$) was observed. The isolate was resistant to most tested

fluoroquinolones and β -lactam antimicrobials except cefepime (< 2 $\mu\text{g/mL}$), ceftriaxone (1 $\mu\text{g/mL}$), and ceftazidime (4 $\mu\text{g/mL}$), and susceptible to amikacin (< 4 $\mu\text{g/mL}$) and gentamicin (< 1 $\mu\text{g/mL}$) (Table 1). Identification of antimicrobial resistance genes displayed the presence of *bla*_{OXA-48}, *fosA5*, and *bla*_{SHV-145}. To the best of our knowledge, this is the first time that we reported *K. pneumoniae* ST16 carrying OXA-48 in China, since an NDM-5-producing *K. pneumoniae* isolate also belonging to ST16 has been previously reported [12], thus, we made an in-depth characterization of this strain.

Whole-genome sequencing data by Illumina MiSeq plus Pacbio found that the *K. pneumoniae* isolate contained a 5.68-Mb genome, including a 5.31-Mb chromosome and seven different plasmids (Table 2). Resistant determinants including *fosA5*, *oqxAB*, and *bla*_{SHV-145} were found in chromosome, whereas, *bla*_{OXA-48} was found in a conjugative IncL/M plasmid (Fig. 1). The size of this plasmid is 66,076 bp, with GC content being 55.23% (Fig. 2). The circular structure of plasmid showed that this plasmid is likely to be a conjugative mobile plasmid because of the frequent binding mobile element *tra*-associated operons region within this plasmid [13] (Fig. 1). As known, IncL/M plasmid has been reported to be the vector for *bla*_{OXA-48} [14]. Analysis of flanking elements of *bla*_{OXA-48} gene displayed mobile elements including insertion sequence 4 (IS4), transposase, and IS_{kra}4. We found that IS4 was distributed among both of the downstream and upstream of *bla*_{OXA-48} gene (Fig. 2). So far, IS4 family element has been reported to be involved in mobilization and expression of β -lactam resistance genes including *bla*_{VEB-1} and *bla*_{OXA-48} [15]. Transposase has been found to mediate chromosomal integration of exogenous genes in *Acidithiobacillus ferrooxidans* [16]. Altogether, our study suggested

Table 2 Genomic features of the OXA-48-producing *Klebsiella pneumoniae*

Structure	Length (bp)	GC (%)	Antimicrobial resistance genes	Replicon type	Accession no
Chromosome	5,314,991	57.49	<i>oqxAB</i> , <i>fosA5</i> , <i>bla</i> _{SHV-145}		CP058581
Plasmid 1	110,716	48.84	None	Unknown	CP058582
Plasmid 2	66,076	51.23	OXA-48	IncL/M	CP058583
Plasmid 3	5,589	51.22	None	Unknown	CP058584
Plasmid 4	5,251	49.24	None	IncCol440II	CP058585
Plasmid 5	5,167	47.57	None	Unknown	CP058586
Plasmid 6	4,693	43.91	None	IncCol440I	CP058587
Plasmid 7	193,269	52.50	Mph(A)	IncFIB(K) IncFII(K)	CP058588

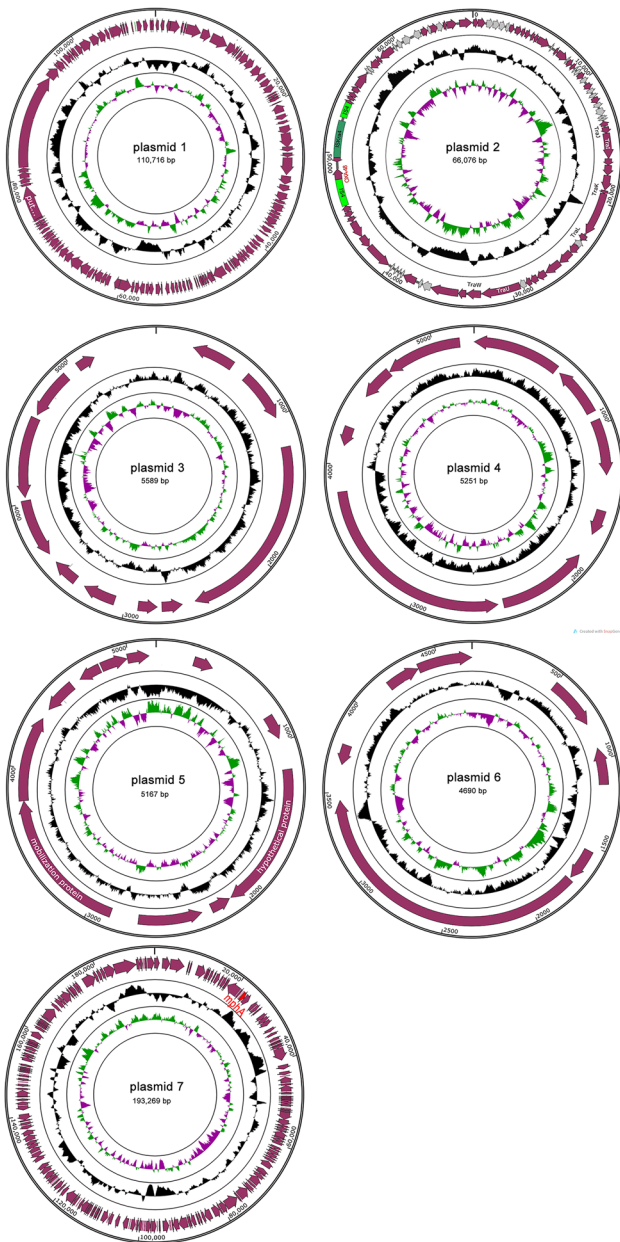


Fig. 1 Circular structure of the seven plasmids in the OXA-48-producing *Klebsiella pneumoniae*. The innermost rings represent the G+C skew of the plasmids, the middle rings represent the G+C content, and the outermost rings represent predicted ORFs. Black, content of G+C; claret, open reading frames; light green, insertion sequence

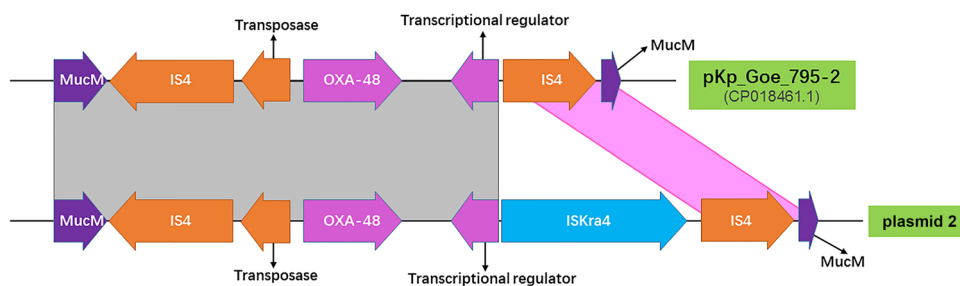


Fig. 2 The flanking element analysis of OXA-48 gene between plasmid 2 and its most similar homologue. Colored arrows indicate open reading frames. Orange,

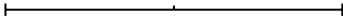
that the *bla*_{OXA-48} gene might be rapidly spread by a broad host-range conjugative plasmid.

Noteworthy, this is the first time that we identified SHV-145 in a clinical *K. pneumoniae* isolate in China. SHV-145 is an extended-spectrum beta-lactamase that has been previously found in a *K. pneumoniae* clinical isolate recovered from a hospitalized patient in Portugal (unpublished data: accession number AFN88952.1). To date, SHV-145 has been predicted by the protein homolog model (AMR detection model) as a beta-lactamase which could lead to the inactivation of penam, cephalosporin and carbapenem (<https://card.mcmaster.ca/ontology/37565#resistomes-table>). Furthermore, SHV-ESBLs are usually encoded by self-transmissible plasmids. However, in our study, *bla*_{SHV-145} was identified in Chromosome, which may be mobilized by mobile elements.

The phylogenetic tree based on the *bla*_{OXA-48} gene from global 576 *K. pneumoniae* isolates displayed 2 clades (Fig. 3). The simple evolutionary relationship suggests that this gene is relatively conservative. Concurrently, this may also indicate that *bla*_{OXA-48} has a strong transmission ability among *K. pneumoniae* isolates.

To the best of our knowledge, this is the first report on the co-occurrence of *fosA5*, *bla*_{SHV-145} and *bla*_{OXA-48} among the *K. pneumoniae* ST16 in China and for the first time, we prescribed the *bla*_{OXA-48} evolutionary phylogenomic of global-producing *K. pneumoniae*.

blue, and purple arrows represent insertion sequence IS4, ISKra4, and *mucM* genes, respectively. Gray shading indicates homologous regions among different elements

Tree scale: 0.1 

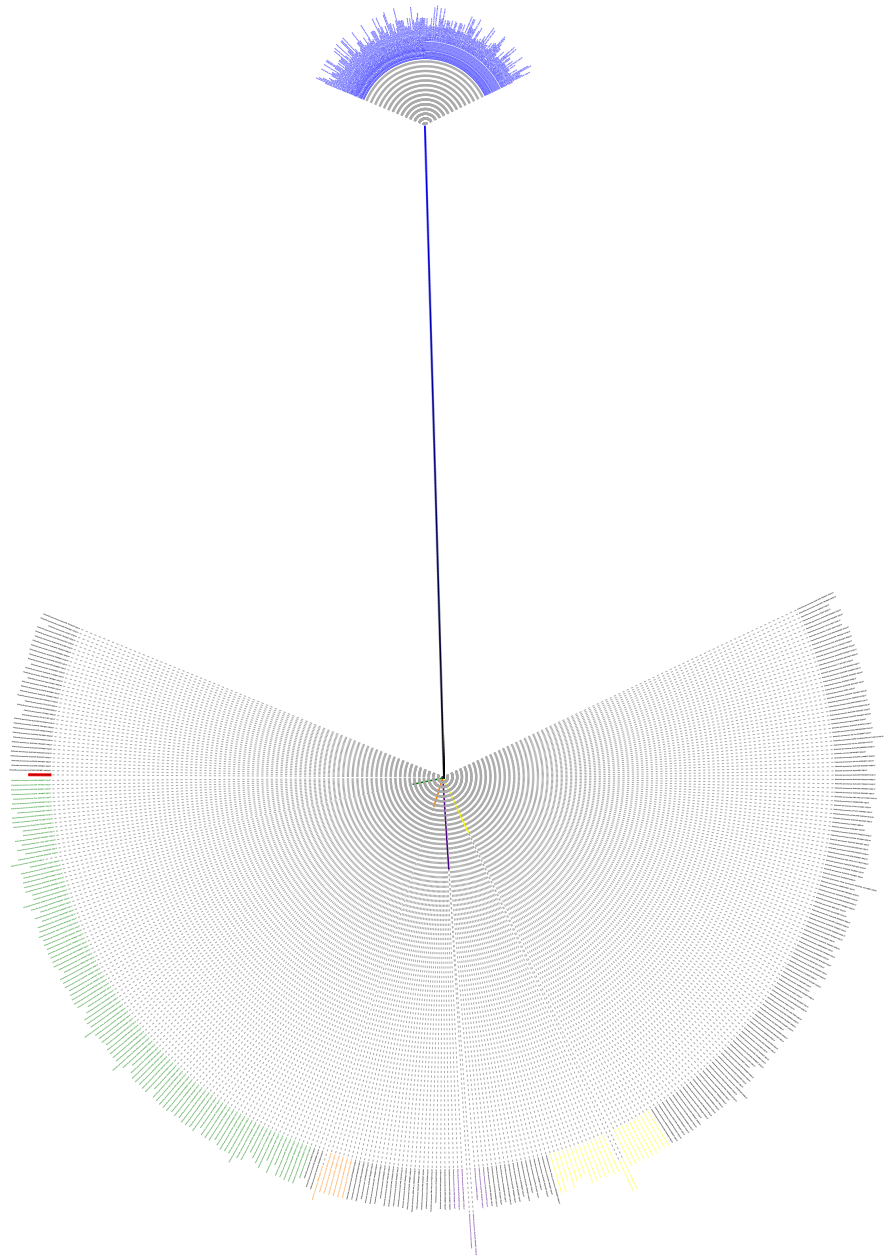


Fig. 3 The bla_{OXA-48} phylogenetic tree based on the global 576 OXA-48-producing *Klebsiella pneumoniae* isolates. The nucleotide sequences of bla_{OXA-48} gene of 576 strains were compared by muscle, and then single nucleotide polymorphism (SNP) sites were used to extract SNPs from mul-

tiple alignment species. The maximum likelihood tree was constructed using RAxML. Red, the OXA-48-producing *K. pneumoniae* isolate in our study

Acknowledgements This research was funded by National Natural Science Foundation of China grants (31770152 and 31400094).

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Poirel L, Heritier C, Tolun V, Nordmann P (2004) Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 48(1):15–22
2. Mairi A, Pantel A, Sotto A, Lavigne JP, Touati A (2018) OXA-48-like carbapenemases producing Enterobacteriaceae in different

- niches. *Eur J Clin Microbiol Infect Dis*: Official Publication of the European Society of Clinical Microbiology 37(4):587–604
3. Han R, Shi Q, Wu S, Yin D, Hu F (2020) Dissemination of carbapenemases (*kpc*, *ndm*, *oxa-48*, *imp*, and *vim*) among carbapenem-resistant enterobacteriaceae isolated from adult and children patients in China. *Front Cell Infect Microbiol* 10:314
 4. Potron A, Kalpoe J, Poirel L, Nordmann P (2011) European dissemination of a single OXA-48-producing *Klebsiella pneumoniae* clone. *Clin Microbiol Infect*: the official publication of the European Society of Clinical Microbiology and Infectious Diseases 17(12):E24–E26
 5. Pitart C, Sole M, Roca I, Fabrega A, Vila J, Marco F (2011) First outbreak of a plasmid-mediated carbapenem-hydrolyzing OXA-48 beta-lactamase in *Klebsiella pneumoniae* in Spain. *Antimicrob Agents Chemother* 55(9):4398–4401
 6. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P (2011) Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. *Antimicrob Agents Chemother* 55(5):2420–2423
 7. Guo L, An J, Ma Y, Ye L, Luo Y, Tao C et al (2016) Nosocomial outbreak of OXA-48-producing *Klebsiella pneumoniae* in a Chinese Hospital: clonal transmission of ST147 and ST383. *PLoS one*. 11(8):e0160754
 8. Lu MC, Tang HL, Chiou CS, Wang YC, Chiang MK, Lai YC (2018) Clonal dissemination of carbapenemase-producing *Klebsiella pneumoniae*: two distinct sub-lineages of Sequence Type 11 carrying *bla_{KPC-2}* and *bla_{OXA-48}*. *Int J Antimicrob Agents* 52(5):658–662
 9. Lu MC, Chen YT, Tang HL, Liu YY, Chen BH, Wang YW et al (2020) Transmission and evolution of OXA-48-producing *Klebsiella pneumoniae* ST11 in a single hospital in Taiwan. *J Antimicrob Chemother* 75(2):318–326
 10. Wayne PA (2020) Performance standards for antimicrobial susceptibility testing, 30th edition, M100Ed30E. Clinical and Laboratory Standards Institute
 11. Alexandros S (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 9:1312–1313
 12. Feng Y, Wei L, Zhu S, Qiao F, Zong Z (2020) Handwashing sinks as the source of transmission of ST16 carbapenem-resistant *Klebsiella pneumoniae*, an international high-risk clone, in ICU. *J Hosp Infect* 104(4):492–496
 13. Burbank LP, Van Horn CR (2017) Conjugative plasmid transfer in *Xylella fastidiosa* is dependent on *tra* and *trb* operon functions. *J Bacteriol* 199(21):e00388-17
 14. Solgi H, Giske CG, Badmasti F, Aghamohammad S, Havaei SA, Sabeti S, et al (2017) Emergence of carbapenem resistant *Escherichia coli* isolates producing *bla_{NDM}* and *bla_{OXA-48}*-like carried on IncA/C and IncL/M plasmids at two Iranian university hospitals. *Infect Genet Evol* 55:318–23
 15. Aubert D, Naas T, Heritier C, Poirel L, Nordmann P (2006) Functional characterization of IS1999, an IS4 family element involved in mobilization and expression of beta-lactam resistance genes. *J Bacteriol* 188(18):6506–6514
 16. Inaba Y, Banerjee I, Kernan T, Banta S (2018) Transposase-mediated chromosomal integration of exogenous genes in *Acidithiobacillus ferrooxidans*. *Appl Environ Microbiol* 84(21):e01381-18

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.