The carbapenem resistance gene blaOXA-23 is disseminated by a conjugative plasmid containing the novel transposon Tn6681 in Acinetobacter johnsonii M19

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Abstract

Background: Carbapenem resistant *Acinetobacter* species have caused great difficulties in clinical therapy in the worldwide. Here we describe an *Acinetobacter johnsonii* M19 with a novel bla<sub>OXA-23</sub> containing transposon Tn6681 on the conjugative plasmid pFM-M19 and the ability to transfer carbapenem resistance.

Methods: *A. johnsonii* M19 was isolated under selection with 8 mg/L meropenem from hospital sewage, and the minimum inhibitory concentrations (MICs) for the representative carbapenems imipenem, meropenem and ertapenem were determined. The genome of *A. johnsonii* M19 was sequenced by PacBio RS II and Illumina HiSeq 4000 platforms. A homologous model of OXA-23 was generated, and molecular docking models with imipenem, meropenem and ertapenem were constructed by Discovery Studio 2.0. Type IV secretion system and conjugation elements were identified by the Pathosystems Resource Integration Center (PATRIC) server and the oriTfinder. Mating experiments were performed to evaluate transfer of OXA-23 to *Escherichia coli* 25DN.

Results: MICs of *A. johnsonii* M19 for imipenem, meropenem and ertapenem were 128 mg/L, 48 mg/L and 24 mg/L, respectively. Genome sequencing identified plasmid pFM-M19, which harbours the carbapenem resistance gene bla<sub>OXA-23</sub> within the novel transposon Tn6681. Molecular docking analysis indicated that the elongated hydrophobic tunnel of OXA-23 provides a hydrophobic environment and that Lys-216, Thr-217, Met-221 and Arg-259 were the conserved amino acids bound to imipenem, meropenem and ertapenem. Furthermore, pFM-M19 could transfer bla<sub>OXA-23</sub> to *E. coli* 25DN by conjugation, resulting in carbapenem-resistant transconjugants.

Conclusions: Our investigation showed that *A. johnsonii* M19 is a source and disseminator of bla<sub>OXA-23</sub> and carbapenem resistance. The ability to transfer bla<sub>OXA-23</sub> to other species by the conjugative plasmid pFM-M19 raises the risk of spread of carbapenem resistance.

Introduction

Carbapenems are considered to be reliable and effective antibiotic agents against most pathogenic bacteria because of their broad antibacterial spectrum [1] and are used in the treatment of serious nosocomial infections caused by cephalosporin-resistant bacteria [1]. Species of the *Acinetobacter* genus are extremely well adapted to the hospital environment and can easily become resistant to available antimicrobial agents; therefore, the isolation of carbapenem-resistant *Acinetobacter* species has raised increasing concerns [2-6]. *A. johnsonii* is an opportunistic human pathogen that colonizes humans but rarely causes clinical infections. Nevertheless, verification of a carbapenem-resistant strain of *A. johnsonii* encoding an extended-spectrum β-lactamase raises concern [7,8].

β-lactamases are common mediators of β-lactam resistance and have been divided into four classes: A, B, C and D [2]. Members of class D, which are also referred to oxacillinases (OXAs), are notable
contributors to carbapenem resistance and have been frequently observed in Acinetobacter species [9]. OXAs with carbapenemase activity were classified into 12 subgroups based on their amino acid sequences [10], and OXA-23 is the major source of carbapenem resistance in Acinetobacter [11].

From the bacterial perspective, conjugative plasmids are an ideal vehicle for transferring resistance genes among species. Fortunately, only a few types of plasmids in Acinetobacter species are conjugative and able to transfer resistance genes into new hosts [12]. However, numerous transposons, such as Tn2006, Tn2007, Tn2008 and Tn2009, have frequently been found to be associated with OXA genes [13]. The migration of OXA genes onto transposons has allowed them to become transmissible factors [14]. In this study, we isolated the high-level carbapenem-resistant strain A. johnsonii M19 from hospital sewage and discovered that it contained a novel transposon in a conjugative plasmid, thus allowing us to explore the potential for dissemination of carbapenem resistance by this species. These results provide new insights into the mechanisms of dissemination of carbapenem resistance.

Materials And Methods

1.1 Isolation and identification of the carbapenem-resistant strain M19

Hospital sewage was obtained from the influx of the wastewater treatment facility in Shandong province, China. The sewage samples were diluted and spread onto Luria–Bertani (LB) agar plates containing 8 mg/L meropenem (Sigma Co. Shanghai, China) and then incubated at 30°C for 24 h. A single clone, named M19, was isolated and cultured in LB medium containing meropenem at 30°C overnight and stored in 15% glycerin at -20°C.

A partial fragment of the 16S rRNA gene of M19 was amplified with the universal primers 27F (5'-agagtttgcctggctcag-3') and 1492R (5'-ggttaccttgttacgactt-3') and sequenced. Similarity analyses of the 16S rRNA sequences were conducted using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was produced by using the neighbor-joining algorithms with the Molecular Evolutionary Genetics Analysis 7 (MEGA 7) software based on BLAST results of the 16S rRNA sequence [15]. Antimicrobial susceptibility tests were performed to determine the MICs for carbapenems based on the breakpoints defined by the Clinical and Laboratory Standards Institute [4].

1.2 Whole-genome sequencing, annotation and analysis

The M19 genome was sequenced by PacBio RS II and Illumina HiSeq 4000 platforms at BGI Co., Ltd. (Wuhan, China). Gene prediction was performed on the M19 genome assembly by glimmer3 (http://www.cbcb.umd.edu/software/glimmer/) with Hidden Markov models [16]. Genome annotation was performed using the Prokaryotic Genome Annotation Pipeline on NCBI (http://ncbi.nlm.nih.govgenome/annotation_prok/). Virulence factors and pathogenicity analysis were
identified based on the core dataset in the Virulence Factors of Pathogenic Bacteria database (VFDB) [17] and the Pathogen Host Interactions (PHI) database [18].

1.3 Bioinformatics analyses of resistance genes, transposon and conjugation system

Antibiotic resistance genes (ARGs) were analyzed by RAST and BLASTp based on the core dataset in the Antibiotic resistance genes database (ARDB) [19]. Multi-sequence comparison was carried out by Clustal Omega [20] and ESPript [21]. Homologous model construction was operated by Discovery Studio 2.0 [22]. Molecular docking was performed by the CDOCKER protocol of Discovery Studio 2.0 [22]. IS transposases were detected by IS-Finder [23]. The new transposon was denominated and registered as Tn6681, according to the Transposon Registry (https://transposon.lstmed.ac.uk/). The genetic context of Tn6681 was compared with Tn2008 and Tn2008B using BLASTn. The conjugation system was identified by PATRIC server [24] and oriTfinder [25].

1.4 Mating experiments

Broth-based mating experiments were carried out using M19 as the donor and Escherichia coli 25DN as the recipient as described previously [26]. M19 and 25DN were cultivated overnight in LB medium containing 8 mg/L meropenem and 220 mg/L sodium azide. The mixture was incubated at 37°C for 30 min, and transconjugants were selected on plates containing 8 mg/L meropenem, 220 mg/L sodium azide and 0.1 mg/L 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid. The conjugal transfer efficiency was calculated, and six transconjugants, named MAT-1 to MAT-6, were isolated and purified. The MICs of carbapenems for these transconjugants were determined as above. To determine whether the plasmid pFM-M19 and blaOXA-23 were transferred to transconjugants, DNA fragments of the plasmid in transconjugants MAT-1 to MAT-6 were extracted and used as templates. The transconjugants were analyzed by PCR with a primer pair (Plasmid-For: 5’-tgtataggtgtgatgccttgta-3’; Plasmid-Rev: 5’-agaaacacagtgatgggagata-3’) for pFM-M19 or a primer pair (OXA23-For: 5’-ctgtcaagctcttaaataatattcagc-3’; OXA23-Rev: 5’-tattcgtcgttagaaaaaattattg-3’) for the blaOXA-23 gene, and DNA sequencing was performed to confirm the presence of blaOXA-23 and plasmid-related genes.

Results

2.1 A. johnsonii M19 has high carbapenem resistance

Strain M19 was isolated from hospital sewage and identified as A. johnsonii based on the 16S rDNA sequence (Fig. 1). MICs of imipenem, meropenem and ertapenem for A. johnsonii M19 were 128 mg/L, 48 mg/L and 24 mg/L, respectively, which were higher than those reported for most A. johnsonii strains (Table 1) [4,27-32], indicating that strain M19 had striking resistance to carbapenems.
The whole genome of M19 was sequenced, and the assembled genome contained one 3.75 Mb circular chromosome with 41.4% GC content, and one 55 kb circular plasmid, here named pFM-M19, with 35.8% GC content. The general features of the complete genome sequence are included in Table S1. Overall, 197 genes (5.24% of the total genes) could be assigned to a VFDB number, and 228 genes (6.07% of the total genes) to a PHI number, indicating that M19 has a high pathogenic potential for humans or other hosts.

Dozens of ARGs were identified in the genome of M19 (Table S2). Three classes of β-lactamase-encoding genes (class B, class C and class D) were identified, including genes encoding six metallo-β-lactamases (MBLs), two AmpCs and two OXAs (OXA-23 and OXA-211). Furthermore, other antibiotic resistance genes, including efflux pumps, a porin and an aminoglycoside-modifying enzyme gene, were also identified.

Insert Fig. 1

Insert Table 1

### 2.2 M19 harbours two oxacillinases genes, \( \text{bla}_{\text{OXA-23}} \) and \( \text{bla}_{\text{OXA-211}} \)

Genome annotation of *A. johnsonii* M19 revealed the presence of two OXA-encoding genes, which are responsible for carbapenem resistance, \( \text{bla}_{\text{OXA-211}} \) in the chromosome and \( \text{bla}_{\text{OXA-23}} \) in plasmid pFM-M19. In addition, \( \text{bla}_{\text{OXA-211}} \) in M19 has the same genetic context conserved in other *A. johnsonii* strains (Fig. S1) and which appears to be ubiquitous in this species [28].

OXA-23 encoded by plasmid pFM-M19 exhibited extremely high amino acid identity with OXA-23 found in *A. baumannii* (CAB69042.1), *A. pittii* (AUF80820.1), *A. wuhouensis* (AY052469.1), *A. indicus* (ANG65640.1), *A. nosocomialis* (AKL90363.1), *E. coli* 521 (AIE13834.1), *A. baylyi* (AER61544.1), *A. radioresistens* (ABX00637.1) and *Klebsiella pneumoniae* (WP_063864531.1) (Fig. S2). M19 OXA-23 also has the conserved active-sites (Fig. S2) (for example Ser-79, Ser-126, Lys-216, Phe-110 and Met-221) that essential to carbapenemase activity [33,34].

The tertiary structure of M19 OXA-23 was modelled based on the crystal structure of 4JF4, which is an OXA-23 from *A. baumannii* (GenBank accession number CAB69042.1) and which had the highest amino acid similarity with M19 OXA-23 in the Protein Data Bank (Fig. 2). In this model, the hydrophobic tunnel was formed by Phe-110 and Met-221, and had an elongated shape. Meropenem, imipenem and ertapenem were able to traverse the hydrophobic tunnel and bound to similar positions in the tunnel (Fig. 2). Additionally, Phe-110, Lys-216, Thr-217, Met-221 and Arg-259 were the conserved reactive amino acids (Fig. S3).

Insert Fig. 2
2.3 \textit{bla}_{OXA-23} is located in the novel transposon Tn\textit{6681} in pFM-M19

To evaluate the potential for horizontal transfer of \textit{bla}_{OXA-23}, the genetic context of \textit{bla}_{OXA-23} was investigated. Notably, sequence analysis found that the region containing \textit{bla}_{OXA-23} formed a composite transposon with the components IS\textit{Aba14-HP-ATPase-bla}_{OXA-23}ΔIS\textit{Aba1-IS\textit{Aba14}} (Fig. 3); this novel transposon has been named Tn\textit{6681} in the Transposon Registry and GenBank (Accession number: MN081614). Further alignment analysis showed that Tn\textit{6681} was highly similar to a chromosomal fragment of \textit{A. baumannii} CBA7, which was isolated in Korea (Accession number: CP020586.1) [35]. However, the \textit{bla}_{OXA-23} context region of CBA7 is IS\textit{Aba10-HP-ATPase-bla}_{OXA-23}IS\textit{Aba1-IS\textit{Aba15}}, which differs somewhat from Tn\textit{6681}. In addition, two IS\textit{Aba14} genes, marked as IS\textit{Aba14}_L and IS\textit{Aba14}_R, were found upstream (3,63,408 bp) of the IS\textit{Aba10} gene and downstream (78,188 bp) of the IS\textit{Aba15} gene in the CBA7 chromosome and share 99.91% identity with IS\textit{Aba14} in Tn\textit{6681} (Fig. 3). Given their overall similarity, we propose that Tn\textit{6681} and this region of the CBA7 chromosome have the same ancestor.

\text{IS\textit{Aba14} genes belong to the IS3 family and have been previously identified as part of the active composite transposon Tn2114 in \textit{A. baumannii} RAB [36]. Analysis of the inverted repeats (IRs) of IS\textit{Aba1} showed that the right inverted repeat (IRR) of IS\textit{Aba1} remained only 9 bp and the direct repeat and inverted repeat sequences vanished, but the left inverted repeat (IRL) of IS\textit{Aba14}_L shared sequence similarity with IRR of IS\textit{Aba14}_R (17/26) in particular the motif TATTT(TG/AT)GCG in their extremities (Fig. 4a). The direct repeat sequences (ATCACTT) of 7 bp were also identified (Fig. 3). This overall structure formed a composite transposon Tn\textit{6681}, which is a novel \textit{bla}_{OXA-23} containing transposon.}

Additionally, the arrangement \textit{ATPase-bla}_{OXA-23}IS\textit{Aba1} constitutes a classic genomic organisation found in Tn\textit{2008} of \textit{A. pittii} (GenBank accession number MF078634) and Tn\textit{2008B} from \textit{A. baumannii} (GenBank accession number LN877214.1) [13]. In Tn\textit{2008}, the promoter of \textit{bla}_{OXA-23} was overlapped by IS\textit{Aba1} upstream of the start codon of OXA-23, and both the -10 and -35 regions of this promoter are within the sequence of the IS\textit{Aba1} gene [37-39]. In Tn\textit{6681}, the insertion of IS\textit{Aba14} into IS\textit{Aba1} generated two ΔIS\textit{Aba1}, but the complete -10 and -35 regions of the \textit{bla}_{OXA-23} promoter were fully maintained (Fig. 4b), indicating that \textit{bla}_{OXA-23} should be expressed normally in M19.

Insert Fig. 3

Insert Fig. 4

2.4 Conjugative plasmid pFM-M19 disseminates \textit{bla}_{OXA-23} and carbapenem resistance

To evaluate the ability to transfer \textit{bla}_{OXA-23} and carbapenem resistance, the conjugation systems of pFM-M19 were analyzed. Components of conjugative machinery were identified in pFM-M19, such as a relaxase; the type IV coupling protein (T4CP) gene (\textit{traG}) for initiation of conjugation; type IV secretion
system (T4SS)-related genes, including the translocation channel protein genes ($trbD$, $trbL$, $trbF$, $trbG$ and $trbI$); the pilus protein genes ($trbC$ and $trbJ$) and the ATPase genes ($trbE$, $trbB$ and $traG$), indicating that pFM-M19 was a conjugative plasmid (Fig. 5a).

Mating experiments between M19 and $E. coli$ 25DN were carried out. The results revealed that the conjugal transfer efficiency of pFM-M19 from M19 to $E. coli$ 25DN was approximately $1.6 \times 10^{-4}$ CFU/donor when 8 mg/L meropenem was used as the selective pressure. The MICs of carbapenems in six transconjugants were 20 mg/L (imipenem), 16 mg/L (meropenem) and 4 mg/L (ertapenem), which were weaker than that of the donor strain M19 but much higher than that of strain 25DN (Table 2). PCR analysis was performed to confirm the dissemination of carbapenem resistance via plasmid pFM-M19 and $bla_{OXA-23}$. The results showed that both pFM-M19 and $bla_{OXA-23}$ were detected in all transconjugants (Fig. 5b and 5c) and suggested that $E. coli$ 25DN obtained carbapenem resistance due to the acquisition of $bla_{OXA-23}$ along with pFM-M19.

Insert Table 2

Insert Fig. 5

Discussion

$A. johnsonii$ strain M19 presented higher carbapenem resistance than that of most $A. johnsonii$ strains [2,4,28-31,40], $Pseudomonas aeruginosa$ [41], $Proteus mirabilis$ [42] or $A. baumannii$ [43]. β-lactam antibiotic resistance of $Acinetobacter$ is mainly due to the inactivation of β-lactams catalysed by four classes (A, B, C and D) of β-lactamases [9,13]. In this work, three classes (class B, C and D) of β-lactamase genes, including six MBL-encoding genes, two AmpC-encoding genes and two OXA-encoding genes, were identified in the genome of $A. johnsonii$ M19, suggesting that M19 is a reservoir of β-lactam resistance genes.

Class D β-lactamases, commonly referred to as OXA, are responsible for carbapenem resistance, and their encoding genes are conserved and widespread in $Acinetobacter$ species [44,45]. M19 harbours $bla_{OXA-211}$ in the chromosome and $bla_{OXA-23}$ in plasmid pFM-M19. OXA-23 was the first reported class D β-lactamase and was originally detected in a patient isolate of $A. baumannii$ in Scotland in 1993 [46]. Twenty years later, the structure of OXA-23 was resolved and revealed that the elongated pocket of OXA-23 provides a hydrophobic environment for high reaction efficiency with carbapenems [11]. OXA-23 is considered to be the major β-lactamase for carbapenem resistance in $Acinetobacter$ [11], suggesting that the OXA-23 encoded by plasmid pFM-M19 plays a key role in carbapenem resistance in M19. Interestingly, the $bla_{OXA-23}$ found in plasmid pFM-M19 has not been previously reported in $A. johnsonii$ strains, indicating that M19 obtained this gene from other bacterial species and further increasing concern over the ability of $bla_{OXA-23}$ to spread among species.
Mobile elements are considered to be responsible for the movement and dissemination of $\text{bla}_{\text{OXA-23}}$, and all of the reported genetic structures that contain $\text{bla}_{\text{OXA-23}}$ have been classified as transposons [13]. Previously, $\text{bla}_{\text{OXA-23}}$ had been found in five transposons, with $\text{ISAba1}$ upstream of the start codon of $\text{bla}_{\text{OXA-23}}$ in four of these (Tn2006 [47], Tn2008 [48], Tn2008B [49], Tn2009 [50]) and with $\text{ISABA4}$ preceding $\text{bla}_{\text{OXA-23}}$ in Tn2007 [47]. In plasmid pFM-M19, we found that $\text{bla}_{\text{OXA-23}}$ was located in the new transposon Tn6681, which has the genetic context $\text{ISAba14-HP-ATPase-bla}_{\text{OXA-23}}$-$\Delta\text{ISAba1}$-$\text{ISAba14}$-$\Delta\text{ISAba1}$ and which was likely formed as two copies of $\text{ISAba14}$ were inserted into the $\text{ISAba1}$ of Tn2008. The structure of Tn6681 has some differences from that of the other transposons previously reported to contain $\text{bla}_{\text{OXA-23}}$ [13]. In addition, in Tn2008, a high level of expression of the $\text{bla}_{\text{OXA-23}}$ gene is associated with significant resistance to carbapenems in A. baumannii, and this expression is controlled by promoter elements within $\text{ISAba1}$ [37-39]. However, although sequence analysis revealed that $\text{ISABA14}$ inserted into $\text{ISAba1}$ in Tn6681, the promoter of $\text{bla}_{\text{OXA-23}}$ appears to be intact, indicating that the $\text{bla}_{\text{OXA-23}}$ gene may still be highly expressed and responsible for the striking carbapenem resistance in M19.

The acquisition of novel genes by plasmids, especially conjugative plasmids [51], along with mobile genetic elements such as transposons or insertion sequences, makes them perfect vehicles for the spread of antibiotic resistance [52]. The conjugative DNA transfer mechanism is well conserved and depends on a T4SS [53,54]. Genes of T4SS and T4CP modules, including genes for a translocation channel protein, pilus protein and ATPase, were identified in pFM-M19, indicating that pFM-M19 is a conjugative plasmid, which has also been confirmed by mating experiments in this study. However, it should be pointed out that the typical origin of transfer site (oriT) was not detected on pFM-M19 by oriT finder, suggesting that pFM-M19 might initiate the transfer from a cryptic oriT. Moreover, the combination of transposon Tn6681 and the conjugative plasmid pFM-M19 may provide a robust means for $\text{bla}_{\text{OXA-23}}$ transfer, with the potential for Tn6681 to shift $\text{bla}_{\text{OXA-23}}$ between the chromosome and plasmid in one bacterial strain, and the conjugative plasmid pFM-M19 disseminating Tn6681 and $\text{bla}_{\text{OXA-23}}$ between different bacterial species (Fig. 6).

**Conclusions**

In conclusion, *A. johnsonii* strain M19, which contains the conjugative plasmid pFM-M19 and Tn6681, is not only a reservoir of $\text{bla}_{\text{OXA-23}}$ but also an effective disseminator of $\text{bla}_{\text{OXA-23}}$. To our knowledge, our investigation is the first to provide evidence that $\text{bla}_{\text{OXA-23}}$ was transferred into *A. johnsonii*. The presence of $\text{bla}_{\text{OXA-23}}$ on conjugative plasmids of *A. johnsonii* enhances the risk of carbapenem resistance spread to the environment and needs to be monitored closely.

**Abbreviations**
MICs: Minimum inhibitory concentrations; PATRIC: Pathosystems resource integration center; OXA: Oxacillinase; LB: Luria–Bertani; MEGA: Molecular evolutionary genetics analysis; VFDB: Virulence factors of pathogenic bacteria database; PHI: Pathogen host interactions; ARGs: Antibiotic resistance genes; ARDB: Antibiotic resistance genes database; MBLs: Metallo-β-lactamases; IRs: Inverted repeats; IRR: Right inverted repeat; IRL: left inverted repeat; T4CP: Type IV coupling protein; T4SS: Type IV secretion system; oriT: Origin of transfer site.

**Declarations**

**Nucleotide sequence accession number**

Complete sequences of the chromosome of *A. johnsonii* strain M19 and of plasmid pFM-M19 were deposited in GenBank under accession numbers CP037424 and CP037425, respectively. The 16S rDNA sequence of strain M19 was deposited in GenBank under accession number MT226917.

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Not applicable.

**Author's contributions**

Gongli Zong conceived and designed and conducted experiments, analyzed and interpreted data, and wrote the manuscript. Yu Zhang, Jiafang Fu, Peipei Zhang and Chuanqing Zhong analyzed data and performed the calculation. Wenchi Zhang and Yan Xu helped to analyze data and revised the manuscript. Rongzhen Zhang and Guangxiao Cao led the project and revised the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article [and its supplementary information files] Original data are available from the corresponding author upon reasonable request.

**Ethics approval and consent to participate**

All activities undertaken did not require Ethics approval. An informed written consent was obtained from the legal guardian of the case.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Lee YR, Baker NT. Meropenem-vaborbactam: a carbapenem and β-lactamase inhibitor with activity against carbapenem-resistant Enterobacteriaceae. Eur J Clin Microbiol Infect Dis. 2018;37(8):1411–19.
2. Zong Z, Zhang X. blaNDM-1-carrying Acinetobacter johnsonii detected in hospital sewage. J Antimicrob Chemother. 2013;68(5):1007–10.
3. Quinteira S, Grosso F, Ramos H, Peixe L. Molecular epidemiology of imipenem-resistant Acinetobacter haemolyticus and Acinetobacter baumannii isolates carrying plasmid-mediated OXA-40 from a Portuguese hospital. Antimicrob Agents Chemother. 2007;51(9):3465–66.
4. Figueiredo S, Bonnin RA, Poirel L, Duranteau J, Nordmann P. Identification of the naturally occurring genes encoding carbapenem-hydrolysing oxacillinases from Acinetobacter haemolyticus, Acinetobacter johnsonii, and Acinetobacter calcoaceticus. Clin Microbiol Infect. 2012;18(9):907–13.
5. Feng Y, Yang P, Wang X, Zong Z. Characterization of Acinetobacter johnsonii isolate XBB1 carrying nine plasmids and encoding NDM-1, OXA-58 and PER-1 by genome sequencing. J Antimicrob Chemother. 2016;71(1):71–5.
6. Octavia S, Xu W, Ng OT, Marimuthu K, Venkatachalam I, Cheng B, Lin RTP, Teo JWP. Identification of AbaR4 Acinetobacter baumannii resistance island in clinical isolates of blaOXA−23-positive Proteus mirabilis. J Antimicrob Chemother. 2020;75(3):521–25.
7. Zong Z. The complex genetic context of blaPER−1 flanked by miniature inverted-repeat transposable elements in Acinetobacter johnsonii. PLoS One. 2014;9(2):e90046.
8. Tian S, Ali M, Xie L, Li L. Genome-sequence analysis of *Acinetobacter johnsonii* MB44 reveals potential nematode-virulent factors. Springerplus. 2016;5(1):986.

9. Lee C-R, Lee JH, Park M, Park KS, Bae IK, Kim YB, Cha C-J, Jeong BC, Lee SH. Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Frontiers in Cellular Infection Microbiology. 2017;7:55.

10. Jeon JH, Lee JH, Lee JJ, Park KS, Karim AM, Lee C-R, Jeong BC, Lee SH. Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. Int J Mol Sci. 2015;16(5):9654–92.

11. Smith CA, Antunes NT, Stewart N, Toth M, Kumarasiri M, Chang M, Mobashery S, Vakulenko SB. Structural basis for carbapenemase activity of the OXA-23 β-lactamase from *Acinetobacter baumannii*. Chem Biol. 2013;20(9):1107–15.

12. Blackwell G, Hall RM. Mobilisation of a small *Acinetobacter* plasmid carrying an *oriT* transfer origin by conjugative RepAci6 plasmids. Plasmid. 2019;103:36–44.

13. Nigro SJ, Hall RM. Structure and context of *Acinetobacter* transposons carrying the *oxa23* carbapenemase gene. J Antimicrob Chemother. 2016;71(5):1135–47.

14. Evans BA, Amyes S. OXA β-lactamases. Clin Microbiol Rev. 2014;27(2):241–63.

15. Jeong S, Hong JS, Kim JO, Kim K-H, Lee W, Bae IK, Lee Kj, Jeong SH. Identification of *Acinetobacter* species using matrix-assisted laser desorption ionization-time of flight mass spectrometry. Ann Lab Med. 2016;36(4):325–34.

16. Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki E, Pruitt KD, Borodovsky M, Ostell J. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res. 2016;44(14):6614–24.

17. Chen L, Dandan Z, Liu B, Yang J, Qin Q, VFDB 2016: hierarchical and refined dataset for big data analysis-10 years on. Nucleic Acids Research, 2016; 44(D1):D694-97.

18. Urban M, Pant R, Raghunath A, Irvine AG, Pedro H, Hammond-Kosack K. The Pathogen-Host Interactions database (PHI-base): additions and future developments. Nucleic Acids Res. 2015;41(D1):D645-D55.

19. Liu B, Pop M. ARDB–antibiotic resistance genes database. Nucleic Acids Res. 2009;37(Database issue):D443-47.

20. Madeira F, Park Ym, Lee J, Buso N, Gur T, Madhusoodanan N, Basutka P, Tivey ARN, Potter SC, Finn RD, Lopez R. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res. 2019;47:W636–41.

21. Xavier R, Patrice G. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res. 2014;42(W1):W320-24.

22. Environment BIOVIA,DStudioM, Release 2017. San Diego, CA: Dassault Systèmes. 2017.

23. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. 2006;34(Suppl 1):D32–6.
24. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M III, Stevens JAT, Vonstein R, Wattam V, Xia AR. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep. 2015;5:8365.

25. Li X, Xie Y, Liu M, Tai C, Sun J, Deng Z, Ou HY. oriTfinder: a web-based tool for the identification of origin of transfers in DNA sequences of bacterial mobile genetic elements. Nucleic Acids Res. 2018;46(W1):W229-W34.

26. Zhang P, Liu M, Fu J, Zhong C, Zong G, Cao G. Identification of a mobilizable, multidrug-resistant genomic island in Myroides odoratimimus isolated from Tibetan pasture. Sci Total Environ. 2020;723:137970.

27. Penzak SR, Gubbins PO, Stratton SL, Anaissie EJ. Investigation of an outbreak of gram-negative bacteremia among hematology-oncology outpatients. Infect Control Hosp Epidemiol. 2000;21(9):597–99.

28. Montaña S, Schramm STJ, Traglia GM, Chiem K, Noto GPD, Almuzara MN, Barberis CM, Vay C, Quiroga C, Tolmasky M, Iriarte A, Ramirez MS. The genetic analysis of an Acinetobacter johnsonii clinical strain evidenced the presence of horizontal genetic transfer. PLoS One. 2016;11(8):e0161528.

29. Rodríguez CH, Nastro M, Dabos L, Barberis C, Vay C, Famiglietti A. First isolation of Acinetobacter johnsonii co-producing PER-2 and OXA-58 β-lactamases. Diagn Microbiol Infect Dis. 2014;80(4):341–42.

30. Maravić A, Skočibušić M, Fredotović Ž, Šamanić I, Cvjetan S, Knezović M, Puizina J. Urban riverine environment is a source of multidrug-resistant and ESBL-producing clinically important Acinetobacter spp. Environ Sci Pollut Res. 2016;23(4):3525–35.

31. Ito A, Sato T, Ota M, Takemura M, Nishikawa T, Toba S, Kohira N, Miyagawa S, Ishibashi N, Matsumoto S, Nakamura R, Tsuji M, Yamano Y. In vitro antibacterial properties of cederocol, a novel siderophore cephalosporin, against gram-negative bacteria. Antimicrob Agents Chemother. 2018;62(1):e01454-17.

32. Radó J, Kaszab E, Benedek T, Kriszt B, Szoboszlay S. First isolation of carbapenem-resistant Acinetobacter beijerinckii from an environmental sample. Acta Microbiol Immunol Hung. 2019;66(1):113–30.

33. Couture F, Lachapelle J, Levesque RC. Phylogeny of LCR-1 and OXA-5 with class A and class D β-lactamases. Mol Microbiol. 1992;6(12):1693–705.

34. Schneider KD, Ortega CJ, Renck NA, Bonomo RA, Powers RA, Leonard DA. Structures of the Class D carbapenemase OXA-24 from Acinetobacter baumannii in complex with doripenem. J Mol Biol. 2011;406(4):583–94.

35. Yoon EJ, Kim JO, Yang JW, Kim HS, Lee KJ, Jeong SH, Lee H, Lee K. The blaOXA−23-associated transposons in the genome of Acinetobacter spp. represent an epidemiological situation of the species encountering carbapenems. J Antimicrob Chemother. 2017;72(10):2708–14.
36. Bonnin RA, Poirel L, Nordmann P. A novel and hybrid composite transposon at the origin of acquisition of \(\textit{bla}_{\text{RTG–5}}\) in \textit{Acinetobacter baumannii}. Int J Antimicrob Agents. 2012;40(3):257–59.
37. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. J Antimicrob Chemother. 2006;57(3):373–83.
38. Choi JY, Ko G, Jheong W, Huys G, Seifert H, Dijkshoorn L, Ko KS. \textit{Acinetobacter kookii} sp. nov., isolated from soil. Int J Syst Evol Microbiol. 2013;63(Pt 12):4402–06.
39. Héritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in \textit{Acinetobacter baumannii}. Antimicrob Agents Chemother. 2005;49(8):3198–202.
40. Krizova L, Maixnerova M, Sedo O, Nemec A. \textit{Acinetobacter bohemicus} sp. nov. widespread in natural soil and water ecosystems in the Czech Republic. Syst Appl Microbiol. 2014;37(7):467–73.
41. Rostami S, Sheikh AF, Shoja S, Farahani A, Tabatabaiefar MA, Jolodar A, Sheikh R. Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant \textit{Pseudomonas aeruginosa} isolated from burn patients. J Chin Med Assoc. 2018;81(2):127–32.
42. Shaker MA, Shaaban MI. Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance: \textit{In vitro} antibacterial study. Int J Pharm. 2017;525(1):71–84.
43. Breilh D, Texier-Maugein J, Allaouchiche B, Saux M-C, Boselli E, Carbapenems. J Chemother. 2013;25(1):1–17.
44. Wong EW, Yusof MYM, Mansor M, Anbazhagan D, Ong G, Sekaran SD. Disruption of \(\text{adeB}\) gene has a greater effect on resistance to meropenems than \(\text{adeA}\) gene in \textit{Acinetobacter} spp. isolated from University Malaya Medical Centre. Singapore Med J. 2009;50(8):822–26.
45. Iacono M, Villa L, Fortini D, Bordoni R, Imperi F, Bonnal RJP, Sicheritz-Ponten T, Bellis GD, Visca P, Cassone A, Carattoli A. Whole-genome pyrosequencing of an epidemic multidrug-resistant \textit{Acinetobacter baumannii} strain belonging to the European clone II group. Antimicrob Agents Chemother. 2008;52(7):2616–25.
46. Paton R, Miles RS, Hood J, Amyes S. ARI 1: \(\beta\)-lactamase-mediated imipenem resistance in \textit{Acinetobacter baumannii}. Int J Antimicrob Agents. 1993;2(2):81–7.
47. Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene \(\text{bla}_{\text{OXA–23}}\) in \textit{Acinetobacter baumannii}. Antimicrob Agents Chemother. 2007;51(4):1530–33.
48. Adams-Haduch JM, Paterson DL, Sidjabat HE, Pasculle AW, Potoski BA, Muto CA, Harrison LH, Doi Y. Genetic basis of multidrug resistance in \textit{Acinetobacter baumannii} clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob Agents Chemother. 2008;52:3837–43.
49. Nigro S, Hall RM. Distribution of the \(\text{bla}_{\text{OXA–23}}\)-containing transposons \text{Tn}2006 and \text{Tn}2008 in Australian carbapenem-resistant \textit{Acinetobacter baumannii} isolates. J Antimicrob Chemother. 2015;70(8):2409–11.
50. Zhou H, Zhang T, Yu D, Pi B, Yang Q, Zhou J, Hu S, Yu Y. Genomic analysis of the multidrugresistant *Acinetobacter baumannii* strain MDR-ZJ06 widely spread in China. Antimicrob Agents Chemother. 2011;55(10):4506–12.

51. Lagarde Md, Larrieu C, Praud K, Lallier N, Trotereau A, Sallé G, Fairbrother JM, Schouler C, BenoîtDoublet. Spread of multidrug resistance IncHI1 plasmids carrying ESBL gene *bla*<sub>CTX–M–1</sub> and metabolism operon of prebiotic oligosaccharides in commensal *Escherichia coli* from healthy horses, France: *bla*<sub>CTX–M–1</sub> plasmids in healthy horses, France. Int J Antimicrob Agents 2020:105936.

52. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. The Journal of Antimicrobial Chemotherapy. 2018;73(5):1121–37.

53. Makart L, Gillis A, Hinnekens P, Mahillon J. A novel T4SS-mediated DNA transfer used by pXO16, a conjugative plasmid from *Bacillus thuringiensis* serovar *israelensis*. Environ Microbiol. 2018;20(4):1550–61.

54. Grohmann E, Christie PJ, Waksman G, Backert S. Type IV secretion in Gram-negative and Gram-positive bacteria. Mol Microbiol. 2018;107(4):455–71.

**Tables**

**Table 1** MICs of carbapenems for the *A. johnsonii* strains
| Strains                        | Carbapenem resistance MIC (mg/L) | References |
|-------------------------------|---------------------------------|------------|
|                              | IPM | MEM | ERP |          |
| *A. johnsonii* M19            | ≥128 | 48  | 24  | This work |
| *A. johnsonii* XBB1           | 4   | ≥2  | /   | [27]      |
| *A. johnsonii* XBC1           | 4   | ≥2  | /   | [27]      |
| *A. johnsonii* Aj306, Aj289, Aj286, Aj205 | ≤1  | ≤0.25 | /   | [28]      |
| *A. johnsonii* CIP70.16       | 0.12 | 0.19 | 3   | [4]       |
| *A. johnsonii* 2199           | 2   | 2   | /   | [29]      |
| *A. johnsonii* 370, 371, 372, 373 | /  | ≥128 | /   | [30]      |
| *A. johnsonii* 363, 366, 364, 367 | /  | 0.25–1 | /   | [30]      |
| *A. johnsonii* ATCC 17909     | /   | 2   | /   | [31]      |
| *A. johnsonii* Z4SZ2          | 0.125 | 0.19 | /   | [32]      |
| *A. johnsonii* ST-2           | 0.75 | 0.38 | /   | [32]      |
| *A. johnsonii* J6             | 0.5  | 0.19 | /   | [32]      |
| *A. johnsonii* 6/1            | 0.5  | 0.38 | /   | [32]      |

Abbreviations: IPM, imipenem; MEM, meropenem; ERP, ertapenem; /, not determined.

**Table 2** MICs of carbapenem-resistant strains
| Strains | MICs (mg/L) |
|---------|-------------|
|         | IPM | MEM | ERP |
| M19     | ≥128 | 48  | 24  |
| 25DN    | <2  | <2  | <2  |
| MAT-1   | 20  | 16  | 4   |
| MAT-2   | 20  | 16  | 4   |
| MAT-3   | 20  | 16  | 4   |
| MAT-4   | 20  | 16  | 4   |
| MAT-5   | 20  | 16  | 4   |
| MAT-6   | 20  | 16  | 4   |

25DN, sodium azide-resistant *E. coli* strain derived from ATCC25922; MAT, transconjugants of *A. johnsonii* M19 and *E. coli* 25DN; IPM, imipenem; MEM, meropenem; ERP, ertapenem.

**Figures**
Figure 1

Neighbor-joining tree generated on the basis of 16S rDNA gene sequences and showing the relationship of M19 to other Acinetobacter species. Bootstrap values are shown as percentages of 1,000 replicates when those values were greater than 50%. The scale bar represents 0.5% substitution per nucleotide position.
Figure 2

Tertiary structure modelling of *A. johnsonii* M19 OXA-23 with carbapenems. (a) Molecular docking models showing embedding of carbapenems in the OXA-23 cavity. (b) Hydrophobicity of OXA-23 surface and hydrophobic tunnel. Meropenem, imipenem and ertapenem are represented, respectively, by red, blue and green stick models.
Figure 3

Linear comparison of structures harbouring blaOXA-23. IRR, right inverted repeat; IRL, left inverted repeat. All block arrows indicate length and orientation. The numbers in each block arrow or box represent the nucleotide sequence identity compared with Tn6681. The distance between ISaba1 or ΔISaba1 and the blaOXA-23 start codon is indicated by the number of base pairs below a curved arrow. Direct repeat sequences are indicated.

a

| IRL ISaba14_L | 1 | 10 | 20 |
|---------------|---|----|----|
| TTTTTCGCCGTAATCTCAAATTTATG |
| IRR ISaba14_R | 34bp | 99.85 |
| TTTATGCCGAAATCTCAAATAGTTG |

b

Extended -10 region

| Tn6681 | CP020586 | Tn2008 | Tn2008B |
|--------|----------|--------|--------|
| 1-20   | 30-40    | 50-60  | 70-100 |
| GTATCCGTCGTTAGAATAACATATTGGTACATTTCAATGATTTCTACTTCATTTTCGTC |
| GTATCCGTCGTTAGAATAACATATTGGTACATTTCAATGATTTCTACTTCATTTTCGTC |
| GTATCCGTCGTTAGAATAACATATTGGTACATTTCAATGATTTCTACTTCATTTTCGTC |
| GTATCCGTCGTTAGAATAACATATTGGTACATTTCAATGATTTCTACTTCATTTTCGTC |

Extended -5 region

| Tn6681 | CP020586 | Tn2008 | Tn2008B |
|--------|----------|--------|--------|
| 1-20   | 30-40    | 50-60  | 70-100 |
| TTTTTCGCCGTTATCCATATTTATAGC |
| TTTTTCGCCGTTATCCATATTTATAGC |
| TTTTTCGCCGTTATCCATATTTATAGC |
| TTTTTCGCCGTTATCCATATTTATAGC |
**Figure 4**

Sequence comparisons of the ISAb1 junction upstream of blaOXA-23. (a) Sequence alignment of left inverted repeat (IRL) of ISAba14L and right inverted repeat (IRR) of ISAba14R. (b) Sequence comparisons of the ISAb1 junctions upstream of blaOXA-23 genes. The start codon of the blaOXA-23 gene is underlined. The -35 and extended -10 regions of the promoter are marked.

![Sequence alignment of ISAb1 junctions](image)

**Figure 5**

Schematic representation of plasmid pFM-M19 and amplification of blaOXA-23 and specific region of pFM–M19 from six transconjugant. (a) The asterisk on ISAb1 indicates a truncated gene. (b, c) M, DNA marker. The following templates were used in PCR: Lanes 1, plasmid fragments extracted from M19; Lanes 2-7, plasmid fragments extracted from the six transconjugants MAT-1 to MAT-6; Lanes 8, genome of 25DN.

![Schematic representation of plasmid pFM-M19](image)

**Figure 6**
Proposed pathway for transfer of the blaOXA-23 gene and dissemination of carbapenem resistance from A. johnsonii M19.