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RESEARCH LETTER

Emergence of a ST248$_{Pasteur}$-ST1068$_{Oxford}$ Carbapenem Resistance *Acinetobacter pittii* Clinical Isolate in China, Co-Harboring OXA-58 and OXA-500 Carbapenemases

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Acinetobacter pittii is a crucial pathogen that mainly causes healthcare-associated infections, including pneumonia, bloodstream infections (BSIs) and urinary tract infections (UTIs).[1] Carbapenem-resistant *A. pittii* (CRAP) poses a huge challenge in clinical settings. CRAP is increasingly recognized as a significant pathogen of hospital-acquired infections in the hospital.[2] Carbapenem resistance in *A. pittii* is mainly caused by the production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) including OXA-23-like, OXA-58-like, and OXA-24-like enzymes.[3] Until now, the genomic features of rare sequence type (ST) *A. pittii* have not been well studied.

Here, one ST248$_{Pasteur}$-ST1068$_{Oxford}$ CRAP clinical strain (TCM2) was collected from sputum in 2018 in China, and its genomic characteristics were described based on Illumina whole-genome sequencing (WGS) and bioinformatic analysis. Antimicrobial susceptibility tests (AST), including imipenem, meropenem, ciprofloxacin, colistin and tigecycline, were conducted using broth microdilutions and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Conjugation experiments were performed using film mating method with a rifampicin-resistant derivative of *A. baumannii* ATCC 17978 as the recipient strain as described previously.[4] Transconjugants were selected on Mueller–Hinton agar plates containing rifampicin (60 μg/mL) and meropenem (4 μg/mL). WGS was performed using the Illumina platform. Sequenced raw reads were assembled using Shovill pipeline, version 0.9. Sequences annotation was performed using National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/) and rapid annotations were conducted using the subsystems technology (RAST) server.[5,6] Multilocus sequence typing (MLST) was performed using Pasteur and Oxford schemes with PubMLST (https://pubmlst.org/). Identification of acquired antibiotic resistance genes using ResFinder database and KmerResistance-2.2.[7,8] Bacterial virulence factors were predicted using virulence factor database (VFDB).[9] Capsular polysaccharide (KL) and lipoolygosaccharide (OCL) were tested using Kaptive.[10] Plasmid replicon analysis was conducted using the *A. baumannii* PCR-based replicon typing (AB-PBRT) method.[11] Comparison between the p17-84_OXA plasmid and TCM2 genome was performed using Circoletto.[12] Closely related plasmids were predicted using the Bacterial Isolate Genome Sequence Database (BIGSdb).[13]

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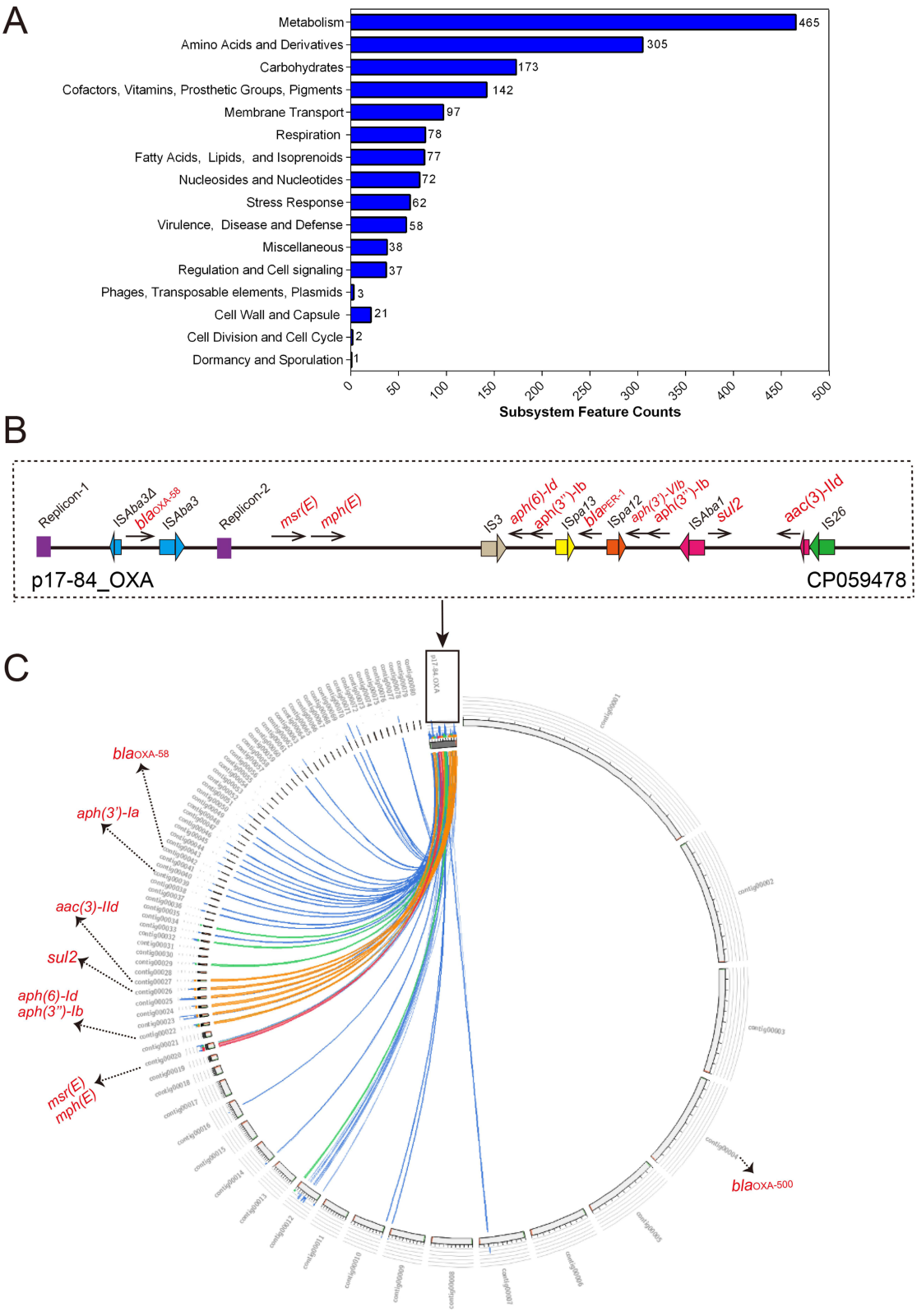

Figure 1 RAST annotation, p17-84_OXA plasmid structure and mapped to the genome of *A. pittii* TCM2 clinical isolate. (**A**) Counts of subsystem based on RAST annotation. The number of each subsystem category is shown on the right of column. (**B**) Structure of p17-84_OXA plasmid (GenBank accession number: CP059478). Resistance genes (black arrows with red names), replicons (purple), and ISs with different colors are shown. (**C**) Comparison between p17-84_OXA plasmid and *A. pittii* TCM2 strain genome. The lines with different colors represent homologous regions with high identity. Resistance genes located in distinct contigs and labeled with red names.

AST results revealed that the TCM2 strain was resistant to imipenem and meropenem, with minimal inhibitory concentrations (MIC) of 8 and 16 mg/L, respectively. It was also resistant to ciprofloxacin (32 mg/L) but was still susceptible to colistin (0.5 mg/L) and tigecycline (0.5 mg/L). According to the RAST results, 3816 genes belonging to 311 subsystems were annotated. Most of these belong to various metabolic functions (465), amino acids, and derivatives (305). In addition, 58 subsystems were sorted into the virulence, disease, and defense categories (Figure 1A). The specific sequence assembly statistics (Size, GC content, N50, L50 and number of contigs) are shown in Supplementary Table 1.

Analysis of the resistance genes profile of *A. pittii* TCM2 strain showed that, in addition to co-harbouring $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-500}}$, a series of genes, including *aac(3)-IId, aph(3")-Ib, aph(3')-Ia, aph(6)-Id, sul2* and *msr*(E)-*mph*(E), were also identified, which was matched with the resistance phenotypes. The results of resistance genes profile were identical using ResFinder database and KmerResistance-2.2. Moreover, a mutation of serine to lysine at amino acid residue 81 (S81L) of the *gyrA*-encoded DNA gyrase confers resistance to fluoroquinolones.[14] In the present study, the putative plasmid was non-typeable by AB-PBRT and no transconjugants were identified for $bla_{\text{OXA-58}}$. Various virulence factors have been predicted in the TCM2 strain, such as pilus-related *csuABCDE*, acinetobactin-encoding gene clusters (*bauBCDEF, basABCDFGHIJ* and *barAB*), and *bfmRS*. Based on genome analysis, *A. pittii* TCM2 was $\text{ST248}_{\text{Pasteur}}$-$\text{ST1068}_{\text{Oxford}}$ with KL84 and OCL6, respectively. We further mapped our genome to the p17-84_OXA plasmid (accession number: CP059478) (Figure 1B), which is located in an ST63 pandrug-resistant *A. pittii*.[15] High nucleotide sequence identity was observed for some of the contigs in the TCM2 genome with p17-84_OXA (Figure 1C). Consequently, we concluded that there was a $bla_{\text{OXA-58}}$-bearing plasmid in the TCM2 strain, which was probably obtained from other *A. pittii* and *A. nosocomialis* strains collected in China in 2012 and 2014, respectively (Supplementary Table 2). Moreover, there is a likelihood that other genes listed above are also plasmid-encoded based on the sequence identities with p17-84_OXA. $bla_{\text{OXA-58}}$ gene was flanked by one truncated IS*Aba3* (upstream) and one complete IS*Aba3* (downstream) with different orientations, which was similar to previous report.[16] Further research of long-read sequencing using Oxford Nanopore Technologies (ONT) platform should be performed to validate the structure of this putative plasmid.

To our knowledge, this is the first report of the genomic characteristics of a $\text{ST248}_{\text{Pasteur}}$-$\text{ST1068}_{\text{Oxford}}$ CRAP clinical isolate that co-produces OXA-58 and OXA-500 in China. Identified resistance genes were correlated with the actual antimicrobial resistance phenotype of the strain and a $bla_{\text{OXA-58}}$-bearing plasmid should be harbored by the TCM2 strain. Therefore, early detection is recommended to prevent further spread in the healthcare settings.

Data Sharing Statement

The draft genome sequence of *A. pittii* TCM2 strain was deposited in GenBank under the Bioproject PRJNA943463, Biosample SAMN33728593, with the accession number JARJKM000000000.

Ethical Approval

Ethical approval was not required due to this study was part of the routine hospital laboratory procedure and this study was only focused on the bacterial genome and not the patient's data.

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Disclosure

The authors report no conflicts of interest in this work.

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