Epidemiological and genomic characteristics of Acinetobacter baumannii from different infection sites using comparative genomics

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Abstract

Background

Acinetobacter baumannii is a common nosocomial pathogen that poses a huge threat to global health. Owing to the severity of *A. baumannii* infections, it became necessary to investigate the epidemiological characteristics of *A. baumannii* in Chinese hospitals and ascertain the reasons for the high antibiotic resistance rate and severe infections. This study aimed to investigate the epidemiologic and genetic characteristics of *A. baumannii* isolated from patients with hospital acquired pneumonia (HAP), bloodstream infection (BSI) and urinary tract infection (UTI) in China and uncover potential mechanisms for multi-drug resistance using whole genome sequencing.

Results

All isolates were classified into one of two primary clades. Clonal complex 208 (CC208) mainly consisted of ST195 (32%) and ST208 (24.6%). CC208 and non-CC208 isolates had carbapenem resistance rates of 96.2% and 9.1%, respectively. UTI isolates possessed the greatest number of unique genes enriched in 'Replication, recombination, and repair' and ‘Amino acid transport and metabolism’ although the numbers of genes specific to HAP-isolates were fewer. No specific virulence gene was identified when comparing isolates from the three infection sites, but most isolates possessed virulence factors related to polysaccharide biosynthesis, capsular polysaccharide synthesis and motility. ABGRI1 antibiotic resistance islands were responsible for streptomycin, tetracycline and sulfonate resistance. The bla_{OXA-23} gene was the most probable cause for carbapenem resistance, although the bla_{OXA-66} gene with nonsynonymous SNPs (F82L, I129L) was not.

Conclusions

Our study illustrates epidemiological and genomic characteristics of *A. baumannii* from HAP, BSI and UTI in China and reveals possible molecular mechanisms of multi-drug resistance. The differential resistance, virulence and genetic features provide supportive evidence for the diverse sites of infection caused by *A. baumannii.*

Background

Acinetobacter baumannii has emerged as a dominant opportunistic Gram-negative bacteria causing a wide range of nosocomial infections [1]. The carbapenem resistance rate of *A. baumannii* reached nearly 75% in response to treatment with imipenem or meropenem in China during 2019 (http://www.chinets.com/). Of particular concern is the mortality rate of ventilator associated pneumonia (VAP) in intensive care units (ICU), which ranges from 45.6–60.9% and even reaches 84.3% when VAP is caused by extensively drug-resistant *A. baumannii* (XDRAB) [2]. Owing to the severe consequences of *A.*

baumannii infection, it is necessary to investigate its epidemiological characteristics and explore potential causes for the high antibiotic resistance rate and severe infections.

Several studies have focused on the molecular epidemiology of clinical *A. baumannii*. In northern China, ST191 and ST195 are the most common sequence types (STs) belonging to clonal complex 92 (CC92, in our study was CC208 as CC92 did not actually exist but several studies have used the original name). All of the *A. baumannii* isolates of these two STs contained carbapenem resistance gene bla_{OXA-23} [3], while ST208 and ST191 are likely the most common STs in southern China [4, 5].

Most comparative genomic studies using whole genome sequencing have focused on the genomic composition of a small number of *A. baumannii* isolates [6]. In our study, differential genetic characteristics were analysed among 64 isolates from hospital acquired pneumonia (HAP), bloodstream infection (BSI), or urinary tract infection (UTI). The epidemiological characteristics, resistance and virulence mechanisms were investigated according to draft genomes. Comparative genomic analysis was conducted in order to identify how these isolates were genetically related and determine the mechanisms behind the different infection locations.

Results

In vitro susceptibility of *A. baumannii* to antimicrobial agents

The resistance rates of clinical isolates of *A. baumannii* against antimicrobial agents are shown in Figure 1. No polymyxins- or tigecycline-resistant isolates were detected. Divided by infection types, isolates from HAP exhibited the highest resistance rates to other antimicrobial agents, while isolates from UTIs showed the lowest. The specific MIC values are presented in Figure 5.

Functional annotation of the genomic sequence of 64 *A. baumanii* isolates

The statistical sequencing information is displayed in Table S1. The full genome length ranged from 3.57 to 4.30 Mb with an average GC content of 38.9%. The pan-genome analysis demonstrated that, with increasing genome number, the slope for the core-genome gene clusters approached an asymptote, whereas the pan-genome gene clusters continued to expand even after the compilation of 64 genomes.

Among 4381 unique gene clusters, 883 (20.2%) in total were annotated into 21 COG terms (Figure 3A). The proportion of core gene clusters was 86.8% (2086/2403). Apart from the ‘poorly characterized’ category, most of the unique genes were annotated into 'Replication, recombination, and repair', 'Cell cycle control, cell division and chromosome partitioning', ‘transcription’ and ‘translation, ribosomal structure and biogenesis’ categories, with the numbers of coding sequences in each being 77, 75, 52 and 57, respectively. These genes mainly took charge of information storage and processing. Meanwhile, unique genes of UTI isolates were more enriched in 'Replication, recombination, and repair', ‘Amino acid transport and metabolism’, ‘Cell wall/membrane/envelope biogenesis’ and ‘Defense mechanisms’. By contrast, the numbers of genes specific to HAP-isolates were much fewer. As for core genes, apart from ‘Translation’

and ‘Transcription’, functions related to metabolism such as ‘Energy production and conversion’ and ‘Inorganic ion transport and metabolism’ were also very crucial (Figure 3B).

Relationship between virulence genes and infection sites

Multiple virulence factors were identified in *A. baumannii* such as *tviB* (related to polysaccharide biosynthesis), *cap8E* (associated with capsular polysaccharide synthesis) and *pilT, pilG* and *pilU,* twitching motility protein. Almost every isolate had at least one twitching motility protein which played a vital role in bacterial invasiveness and colonization. No significant discrepancies in each kind of virulence factor were identified among the three infection sites (Figure S1).

MLST and homology analysis

Sixty-four *A. baumannii* isolates were divided into 11 STs according to the Oxford scheme. ST195 (21/64, 32.8%) was the dominant sequence type followed by ST208 (16/64, 25.0%), ST369 (6/64, 9.38%), ST191 (5/64 7.81%), ST540 (3/64, 4.69%) and ST218 (2, 3.13%). Each of the remaining sequence types (ST761, ST429, ST852, ST605 and ST373) had only one representative isolate. The six new STs were named STnew01 ~ STnew06. Figure 4A displays the distribution of STs across each infection site. The HAP group was mainly composed of ST195 and ST208, while ST208 accounted for most of the BSI group. Isolates from the UTI group were more genetically diverse with 5 new STs. When using the Pasteur scheme, ST2 (53/64, 82.8%) belonging to global clone II (GC2) accounted for the most of the sequence types.

The eBURST analysis depending on Oxford results showed that ST195 was the primary founder (Figure 4B). ST195, ST208, ST369, ST191, ST540 and ST218 (53/64, 82.8% in total) all belonged to clonal complex 208 (CC208, corresponding to GC2). The carbapenem resistance rates of CC208 and non-CC208 isolates were 96.2% and 9.1%, respectively, suggesting that CC208 is a major epidemic clonal complex of carbapenem-resistant *A. baumannii*.

Phylogenetic analysis

The phylogenetic tree is presented in circular (Figure S2) and rectangular configurations (Figure 5). All isolates were classified into two primary clades: CC208 and non-CC208. In the non-CC208 group, 9 of 11 were UTI isolates and 10 of 11 were susceptible. All of the CC208 isolates were carbapenem-resistant with $bla_{\text{OXA-23}}$ gene, except HAP-isolates 080311 and 130811 which demonstrated susceptibility to carbapenems without $bla_{\text{OXA-23.}}$ One UTI-resistant isolate 172315 carried carbapenemase gene $bla_{\text{OXA-58}}$ instead of $bla_{\text{OXA-23}}$. The positive rate of the tetracycline resistance gene *tet (A)* was 71.9%, while that of $bla_{\text{TEM-1}}$ responsible for monobactam resistance was 73.4% (Figure 5).

AbGRI1 antibiotic resistance islands

Intact genomic islands were confirmed in 35 of 64 isolates (Table S2). The smallest island was 9.11 kb and the largest reached 37.8 kb. The GC contents in the islands ranged from 33.7% to 46.8% (median

40.9%), compared to 38.9% of the whole genomes of 64 isolates.

Among the 16 isolates from ST195 (n=4) and ST208 (n=12), 14 aside from 160058 and 130811 had identical Tn*6022△* structure carrying genes *tniA* (transposase), *tniB* (NTP-binding protein), *uspA* (universal stress protein) and *sup* (sulphate permease) (Figure 6). The truncated Tn*5393△* structure with *strA* and *strB* (streptomycin phosphotransferase) was detected in all of the isolates near the 5' end of the *comM* gene. The other genes, such as *tet(B)*, *tetR* and *sul2*, were located in the remaining regions conferring tetracycline and sulfonamide resistance. The genetic structures of the ABGRI1 resistance islands of the other 19 isolates are provided in Table S3.

Relationship between single nucleotide polymorphisms (SNPs) and drug resistance

All identified SNPs were gene mutations with no insertion or deletion detected. Table S4 enumerates all the ns-SNPs that occurred in 64 *A. baumannii*. All of the ns-SNPs in the polymyxin resistance genes are listed in Table 1. The most frequently-occurring ns-SNPs had two amino acid substitutions in the histidine kinase gene *pmrB* which were V9I and I216T. The AA substitution also occurred in functional regions such as I216T in the HisK domain and Q344P in the HATPaseC domain. For the response regulator gene *pmrA*, mutations were discovered in the predicted receiver domain (A39T) and the unknown functional domain (S119T). Lipid A synthesis genes *lpxA* and *lpxC* had one (H131Y) and four (D159N, H149Y, D287N, M115I) amino acid substitutions, respectively, although no polymyxin resistance was detected with MICs ≤ 1 mg/L.

No nucleotide mutation was found in the carbapenemase gene $bla_{\text{OXA-23}}$ and 11 of 12 non-CC208 isolates had the ns-SNPs in $bla_{\text{OXA-66}}$ gene, although these 11 isolates exhibited susceptibility to carbapenems. It is noteworthy that three CC208 isolates belonging to ST369 had the same non-synonymous mutation (F82L, I129L) in $bla_{\text{OXA-66}}$ and displayed resistance to both meropenem and doripenem. The corresponding base change were 244T→C and 385A→C. Nevertheless, in our cloning experiments, the MICs of *E.coli* top10 with mutation 244T→C remained constant or had an ≥ 2-fold decrease compared to wild-type *E.coli* top10. A 2-fold increase of the MICs was observed for *E.coli* top10 with mutation 385A→C. For transformants with both mutations, the MICs remained the same or doubled (Table 2).

Table 1. Amino acid substitutions in polymyxin resistance genes

Amino acid changes							
pmrA[d] (224AA)	*pmrB*[d] (444AA)				*lpxA*		*lpxC*
Rec[a] (AA 5~116)	AA 117~131	AA 1~215	HisK[b] (AA 216~276)	AA 277~330	HATPaseC[c] (AA 331~419)		
A39T	S119T	V9I; K105N; A146V	I216T	\	Q344P	H131Y	D159N; H149Y; D287N; M115I

[a]Rec, signal receiver domain; [b]HisK, histidine kinase (dimerization/phosphoacceptor) domain; [c]HATPaseC, histidine-kinase-like ATPase. [d]Only domains or regions displaying mutations are shown. The amino acid (AA) positions corresponding to these domains are displayed in brackets

Table 2. The susceptibility of *E.coli* top10 and transformants to meropenem and doripenem

Isolate	MIC_{MEM} (mg/L)	MIC_{DOR} (mg/L)
E.coli $top10_{WT}$	0.03	0.03
E.coli $top10_{244T\rightarrow C}$	≤0.015	0.03
E.coli $top10_{385A\rightarrow C}$	0.06	0.06
E.coli $top10_{244T\rightarrow C,385A\rightarrow C}$	0.03	0.06

WT, wild type; MEM, meropenem; DOR, doripenem

Discussion

A. baumannii is a common nosocomial pathogen, mostly causing hospital acquired pneumonia, bloodstream infection and urinary tract infection. However, the genomic characteristics of *A. baumannii* are still unknown for different infection sites. Investigating the epidemiological and genomic characteristics of these isolates is helpful to increase the understanding of the causes for different infections and can further improve clinical therapy. In our study, we investigated the resistance and virulence characteristics of *A. baumannii* from HAP, BSI and UTI, analysing the probable resistance mechanisms and discrepancies for diverse infection sites through whole genome sequencing.

Previous studies have shown that ST195, ST208, ST191 and ST365 are the most widely spread *A. baumannii* sequence types in Chinese hospitals [4, 7]. The dominant types in our study were ST195, ST208, ST218 and ST191. Li [5] collected 52 clinical isolates of *A. baumannii* mainly from sputum as well as bloodstream. The proportion of multidrug and pan-drug resistant isolates in the CC208 group was not significantly different from that in the non-CC208 group. In our study, however, the carbapenem resistance rate of CC208 isolates was significantly higher than that of the non-CC208 group (Fig. 5). Most studies have shown that *A. baumannii* CC208 outbreaks are highly correlated with the presence of the bla_{OXA-23} gene [8]. This was also confirmed in our study, in which 51 carbapenem-resistant isolates from the CC208 group (n = 53) were positive for the bla_{OXA-23} gene. CC208/GC2 was the largest clonal complex in *A. baumannii* which can often carry carbapenemase genes like $bla_{OXA-23-like}$, $bla_{OXA-40-like}$ and $bla_{OXA-58-like}$. Compared to CC109/GC1 and CC187/GC3, CC208 was the only complex carrying three types of hydrolases; this could explain its wide spread to some extent [9].

Comparative genomic analysis demonstrated different genomic characteristics of isolates from different infections. UTI-isolates had a more complex genetic background than isolates from the other two sources. According to COG categories (Fig. 3A), UTI isolates possessed the most unique genes enriched in “Replication, recombination and repair”, “Transcription”, “Amino acid transport and metabolism”, “Cell wall/membrane/envelope biogenesis” and “Defense mechanisms”. This suggests that these unique genes are critical for *A. baumannii* growth and fitness in the urinary tract. Liu [10] and Zhao *et al* [11] also claimed that unique genes may be closely associated with the ability of the bacteria to adapt to challenging niches. HAP-isolates had notably fewer specific genes. The discrepancies in genomic features may affect bacteria colonization in different sites. It also demonstrated that *A. baumannii* is a genomically variable pathogen that has the potential to cause a range of infectious diseases [12].

Apart from the above functional genes, virulence genes played a key role in bacterial fitness and colonization. Multiple factors come together to contribute to the virulence of *A. baumannii* such as biofilm formation, motility, glycosylation and the micronutrient acquisition system [13]. In this study, motility proteins (*pilT, pilU, pilG*) and a polysaccharide biosynthesis protein (*tviB*) seemed to take a leading role in the fitness and colonization success of *A. baumannii.* Subashchandrabose and Wang *et al* investigated about the genes necessary for persistence in the lung and for bacterial survival in bloodstream infection. Seven fitness genes were identified in these two studies, suggesting the presence of a core set of fitness genes irrespective of the site of infection [14, 15]. Although no specific virulence gene was identified among three infection sites, these genes were all identified in the present study.

The multi-drug resistance of *A. baumannii* is a huge threat for clinical treatment and patient health. ABGRI1 resistance islands are a class of vital mobile genetic elements known to be involved in multiple antimicrobial resistance in *A. baumannii* GC2 [16]. *Tn6022* and *Tn6022△* were the most common transposons in AbGRI1. *Tn6022* consists of 7 known functional genes and 2 open reading frames and carries no resistance genes [16, 17]. In our study, the ABGRI1 resistance islands inserted in the *comM* gene shared similar backbones. *Tn6022△* consists of *tniA, tniB, uspA* and *sup* with *tniC, tniD* and *tniE* deleted compared to *Tn6022*. *Tn6022* has been shown to sometimes acquire the OXA-23 carbapenem

resistance transposon *Tn2006* [18], though in our study bla_{OXA-23} may be located on mobile genetic elements like *Tn2009* or *Tn2006* independent of genomic islands [19]. Genes associated with streptomycin, tetracycline and sulfonamide resistance were located on the genomic islands which indicates that ABGRI1 resistance island was not the only contributor to the MDR or carbapenem-resistant phenotype.

Oxacillinases are major causes of carbapenem resistance in *A. baumannii.* Within the CC208 group, the carbapenem-resistant isolates were all positive for bla_{OXA-23}. For the three carbapenem-resistant isolates belonging to ST369 (050711, 051211, 130911), two non-synonymous mutations were both discovered in bla_{OXA-66} gene which referred to F82L and I129L. Previous studies have shown that enzyme OXA-66 can be converted to OXA-83, another subtype in the OXA-51 family, after the substitution of I129L. OXA-83 was first detected in two meropenem-resistant *A. baumannii* strains in the United Kingdom but the values of MIC were both 4 mg/L for imipenem [20]. In terms of tertiary protein structure, Ile-129 was close to the active site Ser-80 and the δ carbon of this isoleucine would cause a steric clash with the hydroxyethyl group of carbapenems [21] that was adverse to substrate binding. I129L relieved this clash, thus promoting carbapenem binding. This has been confirmed by molecular dynamics simulations [22]. When F82L and I129L substitutions both occurred in OXA-66, another subtype OXA-425 was introduced. The OXA-425-positive and carbapenem resistant strain was first isolated in Beijing [23]. To the best of our knowledge, the influence of F82L and I129L on carbapenem resistance has not been confirmed by separate cloning experiments. Our study verified that both of these substitutions failed to cause carbapenem resistance. This suggests that the bla_{OXA-23} gene should be the most likely factor for carbapenem resistance in our *A. baumannii* isolates and mutations of bla_{OXA-66} were not necessarily related to resistance.

The resistance mechanism of *A. baumannii* to polymyxins is mainly regulated by two pathways. One is point mutations of the lipid A synthesis-related genes *lpxA, lpxC* and *lpxD*, which inhibits the synthesis of lipid A [24]. Another mechanism is regulated by the two-component system of *pmrAB*. Studies have shown that point mutations in *pmrB* and the subsequent upregulation of *pmrAB* are critical for polymyxin resistance [25]. Resistance-related point mutations are mainly located in the histidine kinase domain (HisK, AA 216 ~ 276) and the ATP binding domain (HATPaseC, AA 331 ~ 419) of *pmrB* [26]. Resistance caused by point mutations of *pmrA* has also been reported [27], especially in the signal receiver domain (Rec). In this study, several non-synonymous mutations occurred in the *pmrAB* functional domain demonstrating that not all non-synonymous mutations in the *pmrAB* functional region cause resistance. Amino acid changes in the PmrAB two-component system have been suggested to not be essential for *A. baumannii* colistin resistance [28]. Meanwhile, no resistance occurred in the mutants with ns-SNPs in the *lpxA* and *lpxC* genes.

Conclusions

In summary, our study sheds new light on the epidemiological characteristics of clinical *A. baumannii* infection from HAP, BSI and UTI in China and uncovers the possible molecular mechanisms of multi-drug resistance. Our findings indicate that diverse functional genes and sequence types may cause diverse infections.

Methods

Bacterial isolates and antimicrobial susceptibility testing

In this study, clinical *A. baumannii* isolates from BSI (n=17) and UTI (n=16) were collected from Huashan Hospital in Shanghai between 2016 and 2017. Isolates from HAP (n=31) were from a domestic thirteen-centre clinical study on colistin methanesulfonate (registration number: NCT01940731) led by Huashan hospital. Colistin sulfate (lot number SLBD8306V; Sigma-Aldrich, St Louis, MO), polymyxin B (lot number R046V0; USP), minocycline, doxycycline, sulbactam, meropenem, aztreonam (lot numbers: 130514-200401, 130485-201703, 130430-201408, 130506-201403 and 130507-201303, respectively; National Institutes for Food and Drug Control, Beijing, China), tigecycline (lot number: 10-MWC-62-1; USP) and doripenem (lot number 0379; Shionogi & Co Ltd) were used in this study. The minimum inhibitory concentrations (MICs) of 64 isolates were determined using the microbroth dilution method with *Escherichia coli* ATCC25922 as the quality control. A total of nine antimicrobials were included and the results were interpreted referring to the CLSI where possible (CLSI M100 2020). CLSI does not currently provide breakpoints for tigecycline, sulbactam and aztreonam. FDA-recommended criteria for *Enterobacteriaceae* was used for tigecycline susceptibility (≤2, 4, ≥8 mg/L) [29].

DNA extraction and whole genome sequencing

The genome DNA of 64 isolates were extracted according to the Takara DNA Extraction Kit protocol. The whole genome was sequenced using the Illumina Hiseq X10 platform, with the 2*150 bp paired-end sequencing strategy [30]. The raw read data were assembled de novo using Velvet software [31].

Pan-genome profile analysis and functional annotation of genes including resistance and virulence genes

To determine the relationship between pan-genome size and genome number, pan-genome profile analysis was conducted according to pan-genomes analysis pipeline (PGAP) [11].

For functional classification of the predicted genes, BLASTP was used to align amino acids of predicted genes against the Clusters of Orthologous Groups (COG) database with an expected threshold of $1e^{-3}$ using the Conserved Domains Database (CDD) [32]. We also performed sequence alignment of the amino acid sequences to the NCBI non-redundant (NR) database (E-value ≤$1e^{-3}$). Both core and unique genes were categorized based on their COG classification.

In order to identify antibiotic resistance genes, the protein-coding sequences were aligned against the Comprehensive Antibiotic Resistance Database (CARD, https://card.mcmaster.ca/) [33]. The similarity

and length consistency were required to be over 80%. To search possible virulence factors, the Virulence Factors Database (VFDB, http://www.mgc.ac.cn/VFs/) [34] was aligned to the ORF protein sequences, using the same thresholds as alignment of resistance genes. Single nucleotide polymorphisms in genes were investigated including *lpxA, lpxC, pmrA, pmrB* (polymyxin resistance), $bla_{\text{OXA-23}}$, $bla_{\text{OXA-66}}$ (carbapenem resistance), *carO, oprD* (efflux pump), $bla_{\text{Tem-1}}$ (monobactam, penem) and *tet (A)* (tetracycline, glycylcycline). Only non-synonymous SNPs (ns-SNPs) were included in the analysis.

Multilocus sequence typing (MLST) and homology analysis

To determine the sequence types, multi-locus sequence typing (MLST) was performed according to both the Oxford and Pasteur schemes. Sequences were compared to the PubMLST database for *A. baumannii* (http://pubmlst.org/abaumannii/) and then assigned to the appropriate sequence types. Types that were not found in the database were named STnew01, STnew02 and so on.

The eBURST analysis of the isolates was performed to determine their homology [35]. Isolates sharing 6/7 alleles were considered to be a single clonal complex (CC) group.

Phylogenetic analysis

Raw sequence data from all clinical isolates were independently mapped to the reference isolate *A. baumannii* MDR-TJ genome sequence (accession number: NC_017848.1) using bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml). GATK (https://gatk.broadinstitute.org/hc/en-us) was utilized to identify mutations such as SNPs and INDEL. High-quality single nucleotide polymorphisms (SNPs) were selected with a mapping coverage of > 10 and a frequency of > 70%. The multiple sequence alignment was performed using MAFFT and phylogenetic tree was constructed with FastTree following the maximum likelihood method.

ABGRI1 resistance island analysis

The ABGRI1 inserted in the *comM* gene was extracted and analysed. An intact ABGRI1 could be split on different contigs in draft genomes; thus only intact ABGRI1 islands were chosen for further comparative analysis. The annotations were performed with RAST (https://rast.nmpdr.org/) and genomic island structure was constructed using R3.5.0 for isolates belonging to ST195 and ST208.

Cloning experiment

Cloning experiments were conducted to determine the effects of non-synonymous mutations in $bla_{\text{OXA-66}}$ gene on carbapenem susceptibility. Amino acid substitutions was generated using PCR overlap extension [36]. PCR products were ligated into the BamHI and PstI sites of pHSG398 before transforming into the *E.coli* top10. The transformants were selected on Luria–Bertani agar supplemented with 50 mg/L chloramphenicol. The MICs of meropenem and doripenem to transformants were determined and compared to those of wild-type *E.coli* top10.

Accession numbers

Accession numbers of 64 *A. baumannii* are provided in Table S5.

Abbreviations

HAP: hospital acquired pneumonia; BSI:bloodstream infection; UTI:urinary tract infection; CC:clonal complex; XDRAB:extensively drug-resistant *A. baumannii*; MLST:Multilocus sequence typing; SNP:single nucleotide polymorphisms; MIC:minimum inhibitory concentration; AA:amino acid.

Declarations

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Authors' contributions

XCB, XFL, XFZ, JZ and MQF conceived of the study, designed, analysed, and interpreted the data. XL, SCS, HJZ and XL gave assistance in analysing sequencing data. XCB was a major contributor in writing the manuscript.

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Availability of data and materials

The datasets analysed in this study are all available in NCBI (BioProject number PRJNA633416, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA633416).

Ethics approval and consent to participate

The clinical trial (registration number: NCT01940731) has been approved by Huashan Institutional Review Board affiliated to Fudan University. Collecting samples were permitted.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest.

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Figures

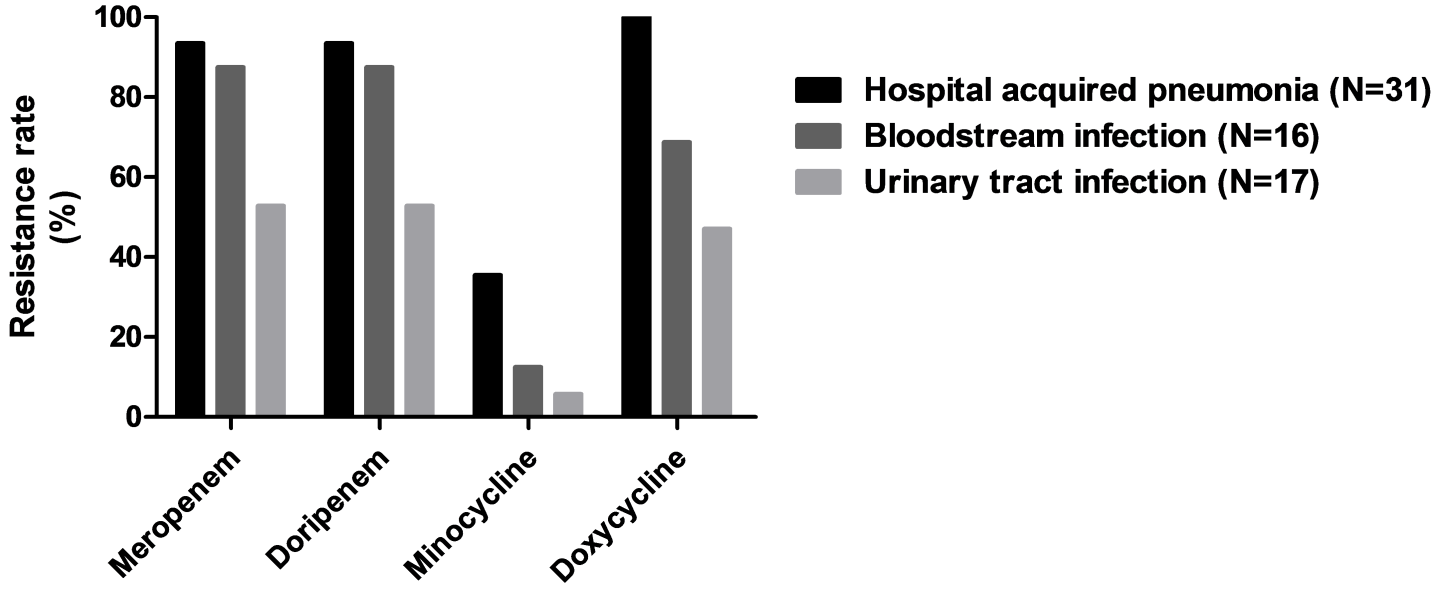

Figure 1

Resistance rates of A. baumannii to antimicrobials. No resistance was detected in colistin, polymyxin B and tigecycline. There is no breakpoint for aztreonam and sulbactam.

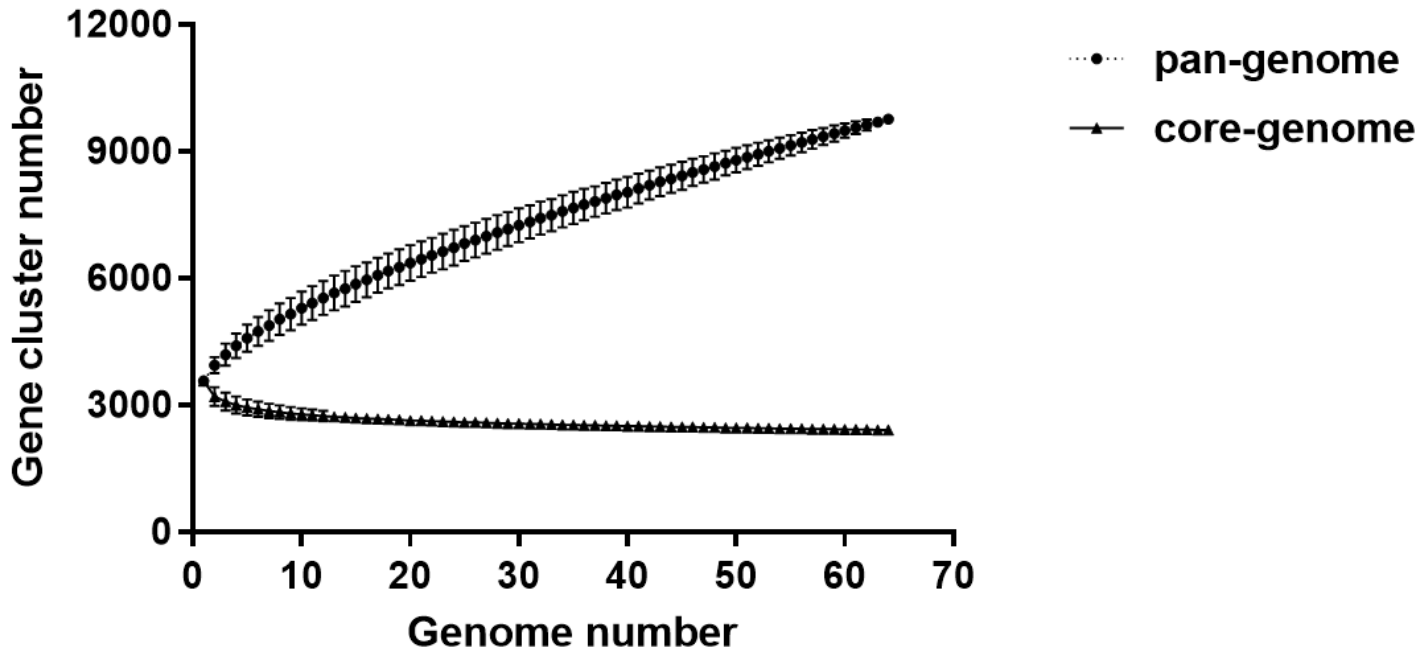

Figure 2

The map for core and pan-genome calculations in 64 A. baumannii isolates

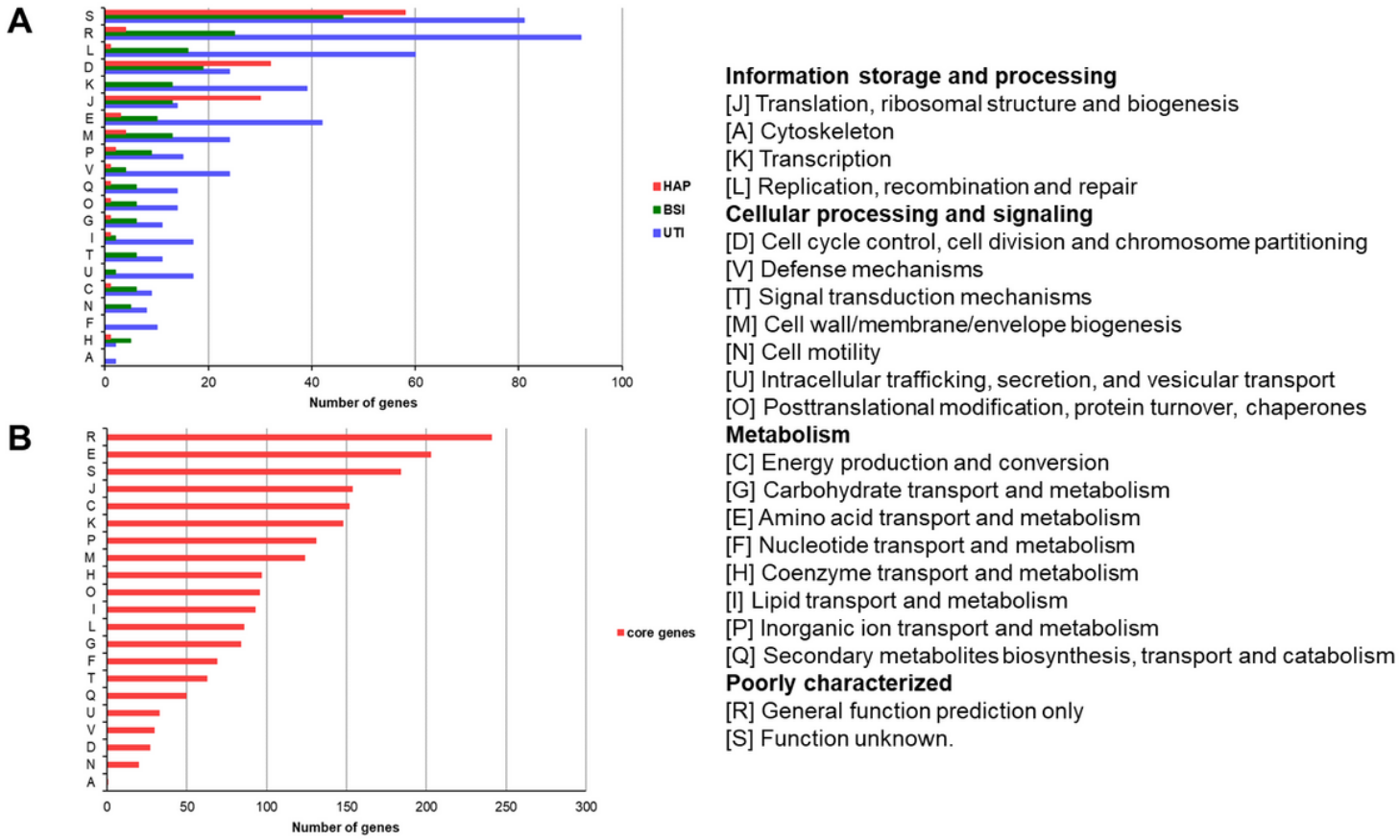

Figure 3

The distribution of COG categories of genomic sequences of A. baumannii. (A) Unique genes of isolates from three infection sites; (B) Core genes of 64 isolates

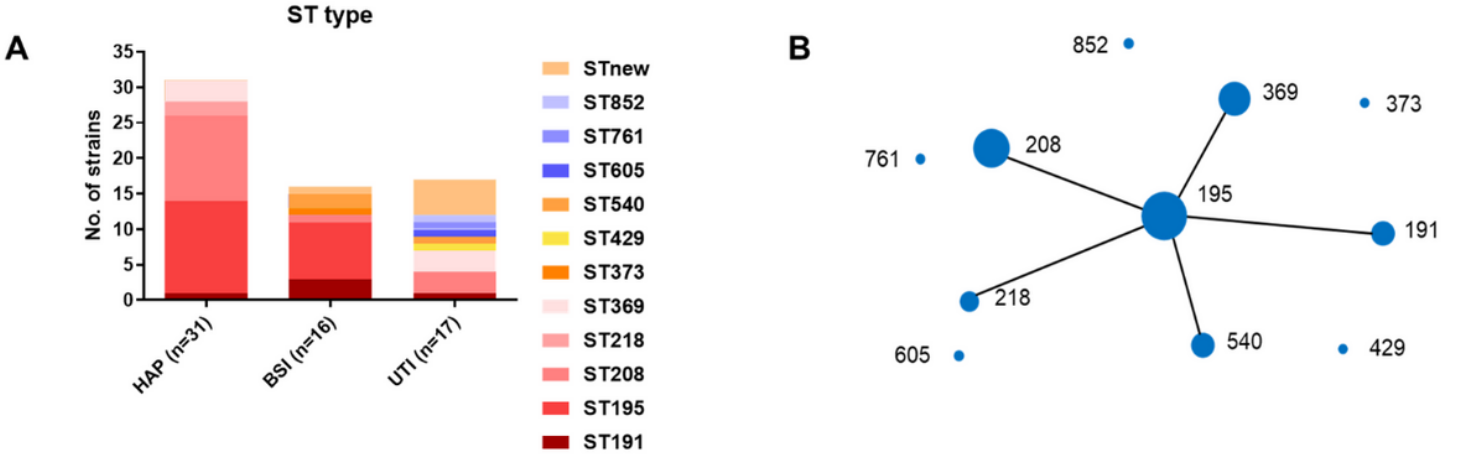

Figure 4

Sequence types of 64 A. baumannii isolates from three infection sites (A). eBURST analysis of 64 A. baumannii isolates (B). Each solid circle represents one sequence type and its size represents the quantity of isolates of this type. Each line between solid circles indicates one allele variation.

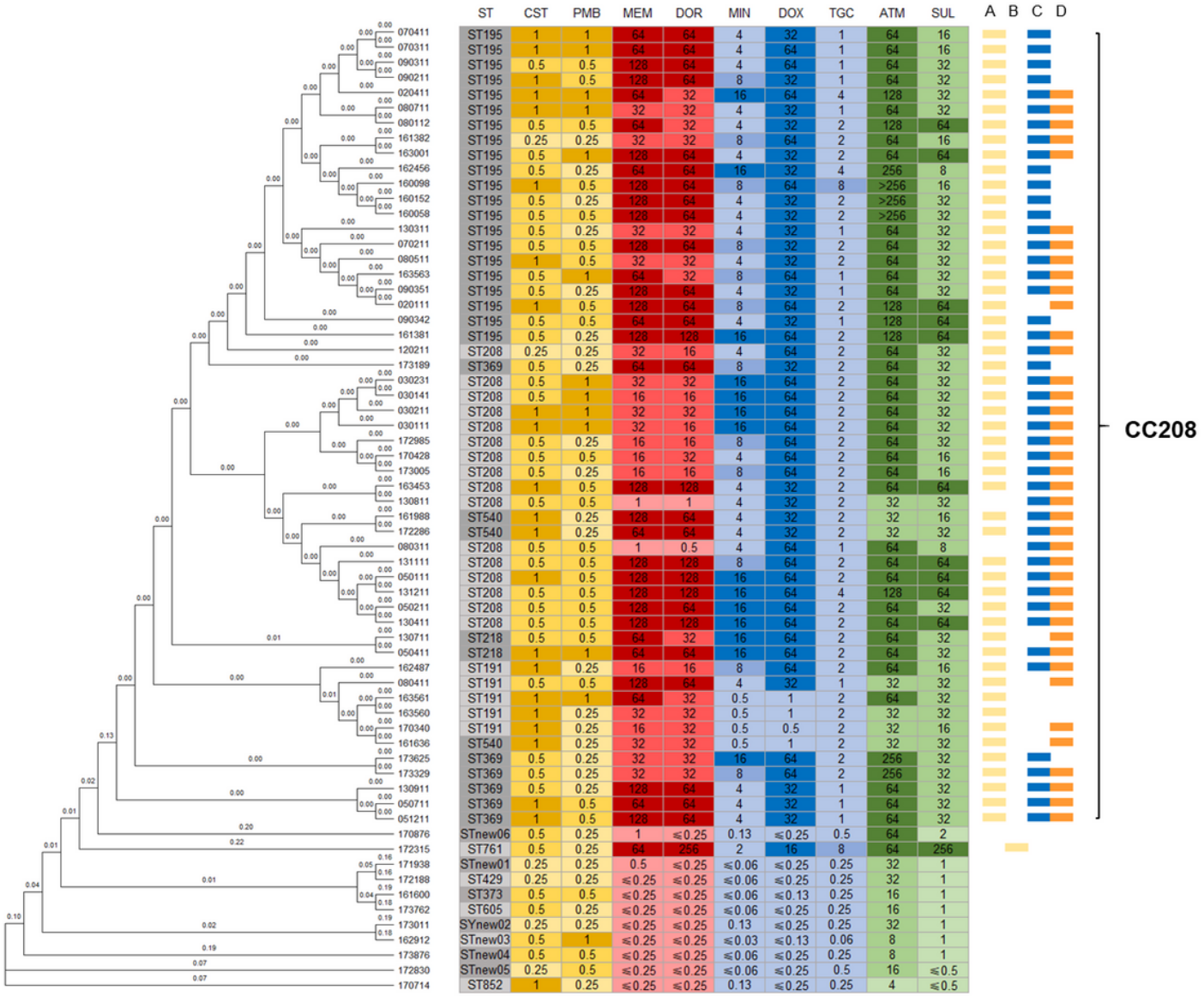

Figure 5

Phylogenetic tree, the relationship among sequence types, resistance phenotype and genotype of 64 A. baumannii isolates. The numbers beside nodes and above the branch represent the bootstrap value and genetic distance, respectively. The darker background color of MIC values indicates high resistance. Note: CST, colistin; PMB, polymyxin B; MEM, meropenem; DOR, doripenem; MIN, minocycline; DOX, doxycycline; TGC, tigecycline; ATM, aztreonam; SUL, sulbactam; A, blaOXA-23; B, blaOXA-58; C, tet (A); D, blaTEM-1.

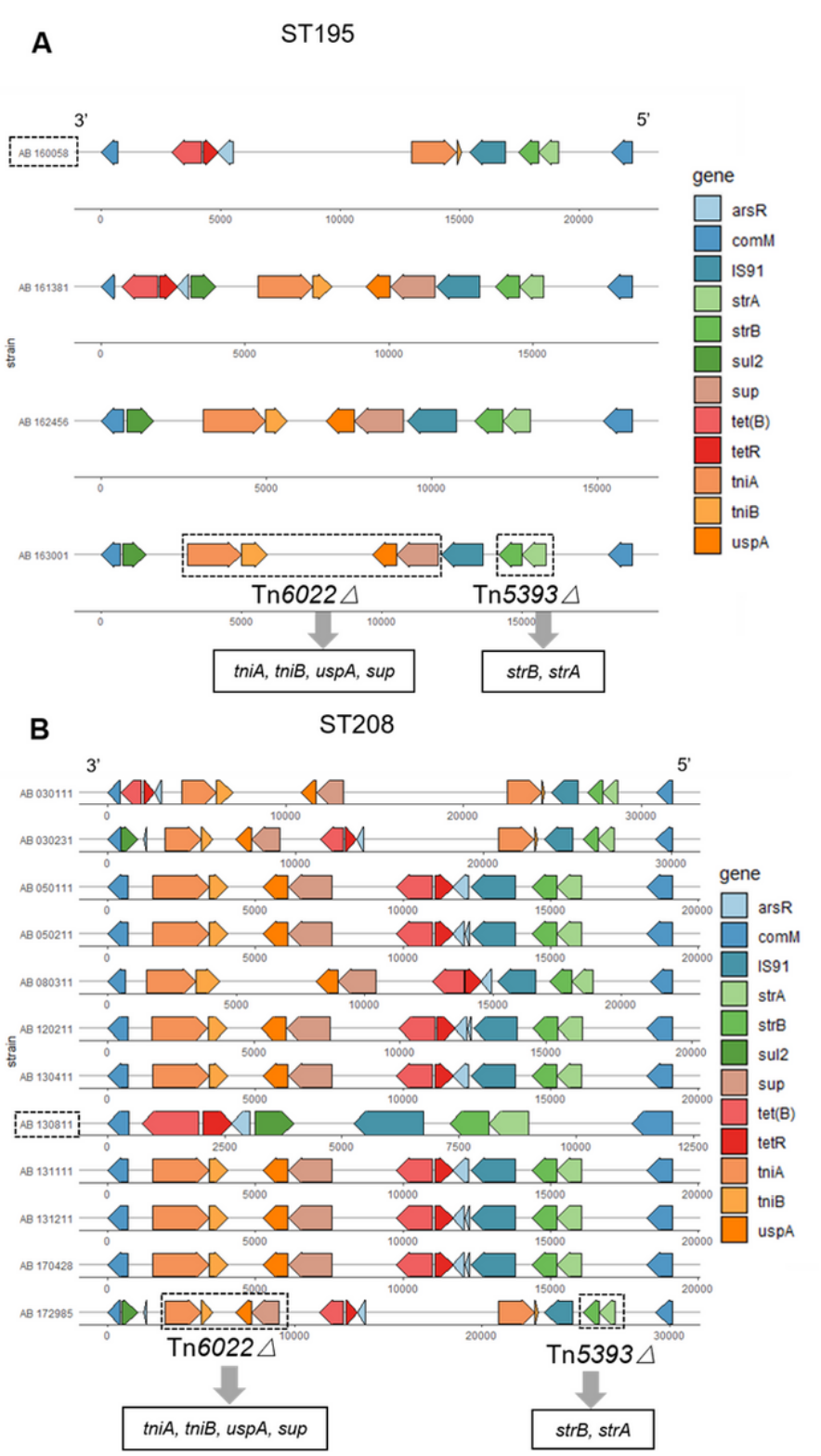

Figure 6

Genetic structures of ABGRI1 resistance islands in 16 strains belonging to ST195 (A) and ST208 (B). arsR, transcriptional regulator; comM, ATPase protein; IS91, insertion sequence 91; strA, strB, streptomycin phosphotransferase; sul2, sulfonamide resistance protein; sup, sulphate permease; tet(B), tetR, tetracycline resistance protein; tniA, transposase; tniB, NTP-binding protein; uspA, universal stress protein A

Supplementary Files

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