

# Genomic Determinants of Pathogenicity and Antimicrobial Resistance of Nosocomial *Acinetobacter baumannii* Clinical Isolates of Hospitalized Patients (2019–2021) from a Sentinel Hospital in Hangzhou, China

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**Purpose:** *Acinetobacter baumannii* (*A. baumannii* or AB) is one of the most opportunistic, nosocomial pathogens threatening public healthcare across countries. *A. baumannii* has become a primary growing concern due to its exceptional ability to acquire antimicrobial resistance (AMR) to multiple antimicrobial agents which is increasingly reported and more prevalent every year. Therefore, there is an urgent need to evaluate the AMR knowledge of *A. baumannii* for effective clinical treatment of nosocomial infections. This study aimed to investigate the clinical distribution AMR phenotypes and genotypes, and genomic characteristics of *A. baumannii* isolates recovered from hospitalized patients of different clinical departments of a sentinel hospital to improve clinical practices.

**Methods:** A total of 123 clinical isolates were recovered from hospitalized patients of different clinical departments during 2019–2021 to analyze AMR patterns, and further subjected to whole-genome sequencing (WGS) investigations. Multi-locus sequence typing (MLST), as well as the presence of antimicrobial-resistant genes (ARGs), virulence factor genes (VFGs) and insertion sequences (ISs) were also investigated from WGS data.

**Results:** The results highlighted that *A. baumannii* clinical isolates had shown a high AMR rate, particularly from the intensive care unit (ICU), towards routinely used antimicrobials, ie,  $\beta$ -lactams and fluoroquinolones. ST2 was the most prevalent ST in the clinical isolates, it was strongly associated to the resistance of cephalosporins and carbapenems, with *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub> being the most frequent determinants; moreover, high carrier rate of VFGs was also observed such as all strains containing the *ompA*, *adeF*, *pgaC*, *lpsB*, and *bfmR* genes.

**Conclusion:** *Acinetobacter baumannii* clinical isolates are mostly ST2 with high rates of drug resistance and carrier of virulence factors. Therefore, it requires measurements to control its transmission and infection.

**Keywords:** *Acinetobacter baumannii*, antimicrobial resistance, genetic determinants, virulence factors, whole-genome sequencing

## Introduction

*Acinetobacter baumannii* (*A. baumannii* or AB) is an opportunistic, gram-negative coccobacillus that widely exists in nature, has the strong adhesive ability and environmental adaptability, and can colonize the human respiratory system or skin/soft tissue surfaces.<sup>1,2</sup> It has the potential to spread among hospitalized patients and is commonly associated with

hospital-acquired nosocomial infections such as respiratory tract infections (RTIs), bacteremia, urinary tract infections (UTIs), secondary meningitis, and surgical site infections.<sup>3,4</sup> Multi-drug resistant *A. baumannii* (MDR-AB) is considered the most prevalent pathogen in the intensive care unit (ICU) environment, and is currently recognized as an emerging cause of nosocomial epidemics worldwide. According to the Infectious Diseases Society of America (IDSA), *A. baumannii* is one of the six most deadly and alarming nosocomial priority pathogens. There are approximately 45,000 cases of *A. baumannii* clinical infections in the United States annually.<sup>5</sup> The mortality rate of *A. baumannii* infections in the intensive care unit (ICU) is as high as 50%.<sup>6</sup>

MDR-AB is one of the most concerning bacteria in terms of control and treatment in both developed and developing countries, raising serious concerns for the MDR crisis.<sup>7–9</sup> Recent studies have revealed the emergence of MDR bacterial infections in humans, birds, cattle and fish.<sup>10–12</sup> Consequently, this has increased the necessity for routine antimicrobial susceptibility testing (AST) to find the recommended antibiotics and screen for the emergence of MDR strains. MDR pathogens resistant to various antimicrobials, including  $\beta$ -lactams, fluoroquinolones, tetracyclines, and aminoglycosides. Several virulence factor genes (VFGs) responsible for *A. baumannii* pathogenicity have been found, including outer membrane porins, pilus, lipopolysaccharides, capsular polysaccharides, phospholipases, protein secretion systems, and iron-chelating systems. Some clinical strains share genes that are involved in adhesion, invasion, and survival, and they can form biofilms.<sup>13,14</sup> Importantly, *A. baumannii* forms biofilms on polystyrene and glass as well as on fungal filaments and epithelial cells. In addition, the adhesion and biofilm phenotypes of few clinical isolates were associated with the presence of broad-spectrum antibiotic resistance.<sup>15</sup> Due to the high prevalence of infections and the shortage of effective antibiotics for treatment, *A. baumannii* is one of the most problematic pathogens causing nosocomial infections. To address this issue, it is important to identify antimicrobial resistance (AMR) and the VFGs determinants in clinical isolates.

The progress in whole genome sequencing (WGS) technology has promoted research on bacterial typing, whole genome characteristics, drug resistance mechanisms and pathogenesis of *A. baumannii*.<sup>16,17</sup> The rapid development of this technology, accompanied by a significant reduction in price, has enabled WGS to be widely adopted, with over 3500 *A. baumannii* genomes available by early April 2019.<sup>18</sup> However, *A. baumannii* genome information has distorted geographical distribution, with 69% (2481/3575) of all sequenced strains across the five countries (the United States, the People's Republic of China, Thailand, Australia, and Pakistan). The analysis of the genome sequences revealed that ST2, ST1, ST79, and ST25 account for more than 71% of all the genomes that have been sequenced to date, with ST2 being the most widespread type.<sup>18,19</sup>

Nevertheless, our previous investigations have demonstrated the routine use of WGS technology for studying molecular epidemiology, AMR pattern, MLST types, VFGs and the correlation between VFGs and AMR profiles.<sup>20–23</sup> Therefore, WGS technology was used to investigate the genomic features of 123 *A. baumannii* clinical isolates recovered from 2019 to 2021 from the specimens of hospitalized patients from different clinical departments of the First People's Hospital in Hangzhou, Zhejiang province, China. In addition, we also statistically analyzed the clinical distribution, AMR phenotypes and genotypes, and the relationship between clinical diagnosis and antimicrobial susceptibility testing (AST) of isolates. Therefore, this comprehensive and integrated research study could improve our understanding regarding AMR pattern in clinical *A. baumannii* isolates and provide essential knowledge to clinicians for clinical therapy and management as well as pinpointing the nosocomial infections.<sup>24,25</sup>

## Materials and Methods

### Bacterial Isolation and Identification of *Acinetobacter baumannii*

A total of 123 isolates were recovered from specimens from different clinical departments of the First People's Hospital in Hangzhou, Zhejiang Province, China, during the three years (2019–2021). All clinical specimens from patients were inoculated onto blood agar plate and incubated at 37°C for 18–24 hrs. The dominant growing strains were transferred to another blood agar plates for pure culture for 18–24 hrs, followed by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) (Bruker Microflex LT/SH, Billerica, Massachusetts, USA) and antimicrobial susceptibility testing (AST). The identified *A. baumannii* strains were stored at –80°C using glycerol with a final concentration of 20%

(v/v). The isolates were resuscitated when used and re-identified by mass spectrometry. Each patient was matched to one specimen, and each specimen was matched to one strain of *A. baumannii*. The clinical data of 123 patients were statistically analyzed, and the distribution of ages, genders, clinical diagnoses, and treatments was determined.

## Antimicrobial Susceptibility Testing

In this study, the phenotypic antimicrobial resistance (AMR) of *A. baumannii* isolates was evaluated by automated identification/susceptibility testing system bioMérieux (VITEK2 Compact, Durham, USA) and Kirby-Bauer disc diffusion method as recommended by CLSI guidelines (CLSI, 2019). The minimum inhibitory concentrations (MICs) and zone size/diameters for all isolates were determined against twelve antibiotics belonging to six antimicrobial classes according to the recommendations of the Clinical Laboratory Standards Institute guidelines (CLSI, 2019).<sup>26</sup> *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality control strains to validate antimicrobial susceptibility testing (AST) as recommended by the CLSI. Isolates were nonsusceptible to  $\geq 1$  agent in  $\geq 3$  antimicrobial classes were considered multi-drug resistant (MDR).<sup>27,28</sup> The tested antimicrobials and their detection concentration are mentioned in [Table S1](#).

## DNA Isolation, Whole Genome Sequencing (WGS), and Genome Assembly

Genomic DNA was extracted from isolates using the Vazyme Bacterial DNA Kit (Vazyme Biotech, Nanjing, China) according to the manufacturer's recommended protocol. The extracted DNA was quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, California, USA) according to the manufacturer's instructions. Whole-genome sequencing (WGS) was performed by next-generation sequencing (NGS) using the Illumina NovaSeq platform (San Diego, California, USA) and the NovaSeq 6000 SP kit (300 cycles). The quality of the whole-genome library was checked by quality control. The sequence data were trimmed with Trimmomatic v0.39. De novo assemblies of our 123 *A. baumannii* genomes were generated using SPAdes v3.12.0.<sup>29</sup>

## Bioinformatics Analysis

Sequence analyses were performed using several bioinformatics tools. The sequence types (STs), antimicrobial-resistance genes (ARGs), and plasmid types were identified using assemblies of the samples on the Galaxy platform,<sup>30,31</sup> combined with mlstv2.16.1 (<https://github.com/tseemann/mlst2016>) and ABRicate v1.0.1 (<https://github.com/tseemann/abricate>),<sup>32</sup> including ResFinder v3.2 (<https://cge.cbs.dtu.dk/services/ResFinder/>) with a 90% similarity cutoff for ARGs.<sup>33–35</sup> The plasmid replicons were identified using PlasmidFinder database (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) with a similarity cutoff of 95%.<sup>36</sup> The virulence factor genes (VFGs) were identified using virulence factors database (VFDB).<sup>37</sup> The presence of insertion sequence (IS) elements was predicted using ABRicate v1.0.1 with the default parameter against the IS database from ISfinder (<https://www-is.biotoul.fr/>).<sup>38</sup> In addition, single nucleotide polymorphisms (SNPs) alignment was matched using package Snippy v4.4.4, and phylogenetic trees were constructed by IQ-tree v1.6.12 and TVM+F+R3 models. Interactive Tree of Life (iTOL) v6 software (<https://itol.embl.de/>)<sup>39</sup> was to visualize and edit the phylogenetic tree.

## Statistical and Data Projection Analysis

Clinical data were analyzed using SPSS software Version 19.0 (IBM SPSS Inc., Chicago, USA), and the figures of STs, AMR profiles and VFGs were generated by GraphPad Prism 8 (San Diego, CA). The distribution of various STs and genetic relatedness of *A. baumannii* clinical isolates are illustrated in the minimum spanning tree (MST) using PHYLOViZ software 2.0 for providing scalable data integration and visualization according to the goeBURST algorithm.<sup>40</sup> Pearson correlations ( $r$ ) among AMR genotype, phenotype, VFGs and STs of *A. baumannii* clinical isolates were determined using R software. Binary data (0/1) were imported into R software (version 4.1.0, R Foundation for Statistical Computing, Austria), and correlations were determined using the “cor” function and visualized using the “ggcorplot” function. Correlation significance ( $P < 0.05$ ) was also determined by the “cor.mtest” function. The correlation is strong if  $r \geq 0.7$ , moderate if the  $r$  value is between 0.4 and 0.69, and weak if  $r < 0.4$ .<sup>41</sup>

## Results

### Distribution of *Acinetobacter baumannii*

The distribution of clinical *A. baumannii* isolates in different clinical departments recovered or isolated from various clinical specimens is shown in the Table 1. The prevalence of *A. baumannii* isolates was high in intensive care units (ICU) (62.60%, 77/123), followed by the internal medicine department. The respiratory system was the most common body site for *A. baumannii* infections (86.18%, 106/123), and all specimens isolated from blood (7.32%, 9/123) were from the ICU department.

The *A. baumannii* isolates were obtained from 123 clinically infected patients, of which 82 cases (64.1%) were males, and 41 cases (33.3%) were females. Hence, a gender ratio (M/F) was 2:1. Centralized trend indicators showed that the median age of 82 males was 71 years (interquartile range: 14–96 years) and the median age of 41 females was 76 years (interquartile range: 27–97 years), and the distribution of age showed that 1/4 of the population was younger than 62 years, 1/2 was younger than 73 years, and 1/4 was older than 81 years. In addition, 70% of the patients were between 62 and 89 years, predominantly middle-aged and older adults.

Among the 96 patients with clear clinical diagnosis and treatment information (27 patients with Incomplete treatment information) were counted, the clinical diagnosis was dominated by pulmonary infections (88.54%, 85/96) followed by bloodstream infections (9.37%, 9/96). In terms of clinical treatment, enzyme-inhibitor complexes were the predominant agents, in which penicillin complexes and cephalosporin complexes account for 35.42% (34/96), 23.96% (23/96), respectively (Table 2).

### Antimicrobial Susceptibility Testing

The antimicrobial resistance profiles of all 123 clinical *A. baumannii* isolates against twelve antibiotics belonging to six antimicrobial classes are shown in Figure 1 and Table S2. The results showed a high resistance rate towards  $\beta$ -lactams

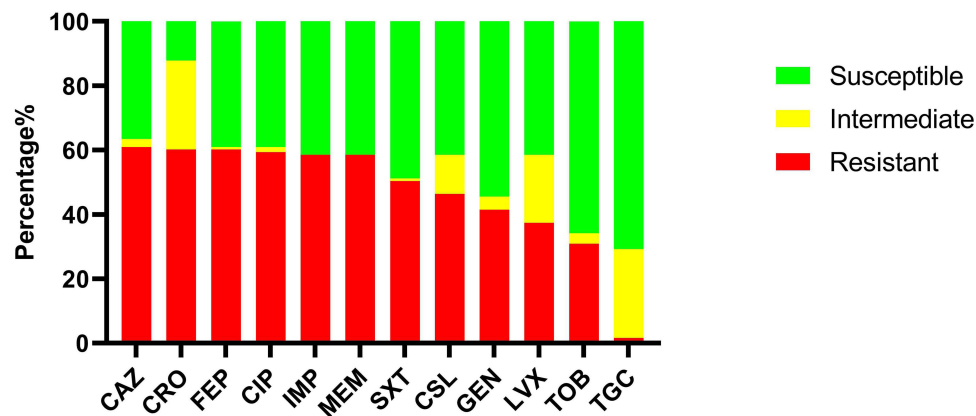
**Table 1** Distribution of *Acinetobacter baumannii* Based on Mode of Acquisition of Infection and in Various Clinical Specimens

Clinical Departments	Clinical Specimens					Total
	Blood	Respiratory	Urine	Bile	Pus	
Internal Medicine	0	36	3	1	0	40
Surgery	0	4	1	1	0	6
ICU	9	66	1	0	1	77
Total	9	106	5	2	1	123

**Table 2** Correlation Between the Clinical Diagnosis and Antibiotics Used in the Infected Patients

Diagnosis	Cephalosporins	Antibiotics				Total
		Cefoperazone/ Sulbactam	Piperacillin / Tazobactam	Carbapenems	Co-Medication	
Pulmonary infection	11 (11.46%)	23 (23.96%)	34 (35.42%)	15 (5.63%)	2 (2.08%)	85 (88.54%)
Biliary tract infection	1 (1.04%)	0	0	0	0	1 (1.04%)
Bloodstream infections	0	2 (2.08%)	0	1 (1.04%)	6 (6.25%)	9 (9.36%)
Wound infection	0	0	0	0	1 (1.04%)	1 (1.04%)
Total	12 (12.5%)	25 (26.04%)	34 (35.42%)	16 (16.67%)	9 (9.38%)	96

**Notes:** Cephalosporins: ceftriaxone, cefdinir, cefotaxime, ceftazidime, ceftizoxime. Cephalosporin enzyme inhibitors: cefoperazone-sulbactam. Carbapenems: meropenem, imipenem, ertapenem. Penicillin enzyme inhibitors: piperacillin-tazobactam. Co-Medication refers to the simultaneous use of two or more antibiotics. Such as cephalosporins + quinolone, cephalosporins + lincosamide, cephalosporins + carbapenem, cephalosporin + peptides, cephalosporins + penicillins, cephalosporin + tetracyclines, peptides + tetracyclines + cephalosporins. The figures (n) represents the number of patients, and the percentage (%) represents the number of patients/total number of patients treated. The antibiotics listed in Table 2 are the therapeutic drugs after *Acinetobacter baumannii* was detected and according to the results of antimicrobial sensitivity testing.



**Figure 1** Distribution of antimicrobial resistance (AMR) pattern of 123 *Acinetobacter baumannii* clinical isolates.

**Notes:** The AMR pattern of resistant isolates after antimicrobial susceptibility testing of all isolates (n=123). The percentage (%) is calculated by the number of each antibiotic resistance, intermediate or susceptible isolate/total number of isolates.

**Abbreviations:** CRO, ceftriaxone; CSL, cefoperazone-sulbactam sodium; IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; CAZ, ceftazidime; FEP, cefepime; GEN, gentamicin; TOB, tobramycin; TGC, tigecycline; SXT, cotrimoxazole.

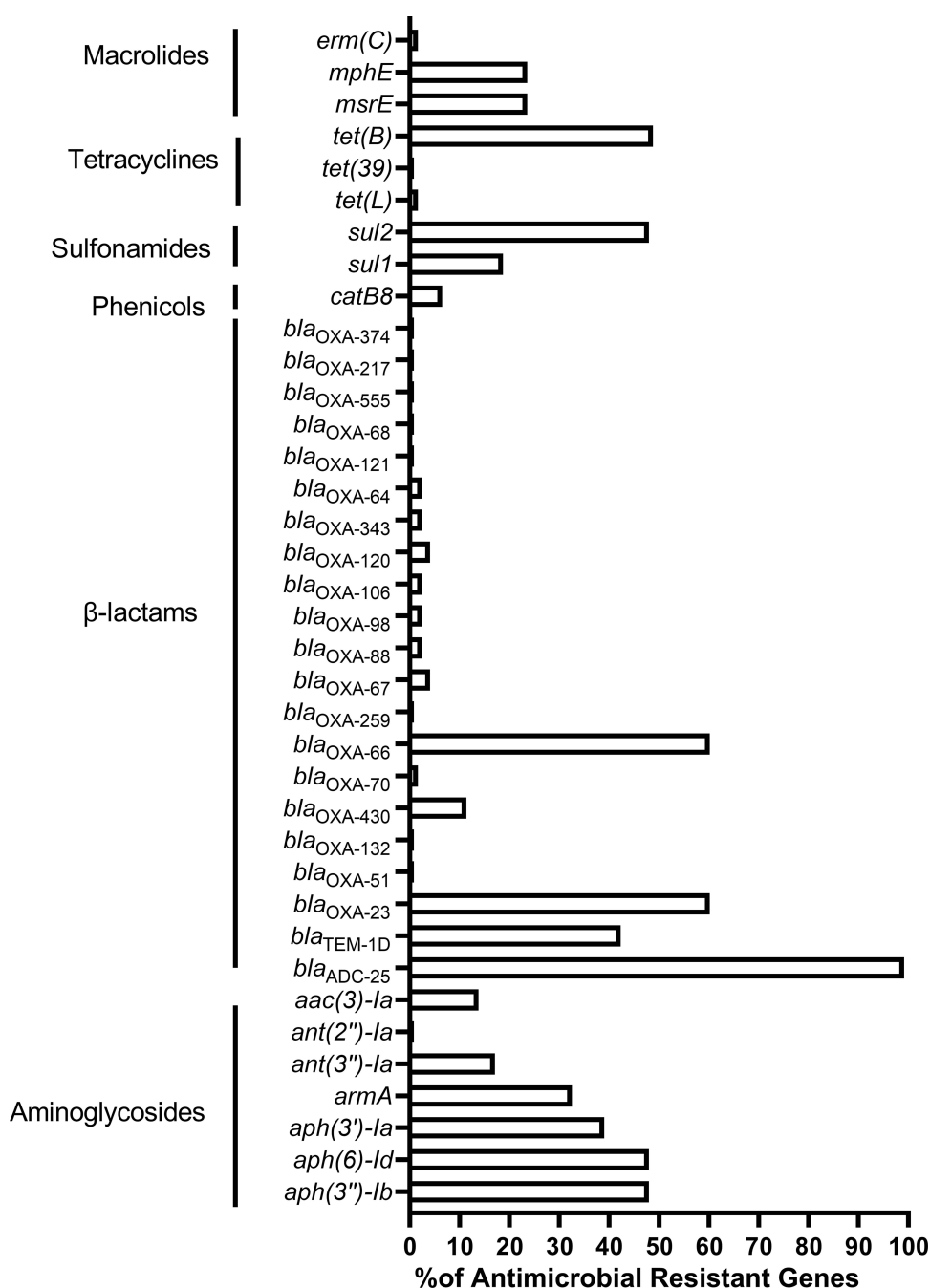
(cephalosporins), with ceftazidime (60.97%, 75/123), ceftriaxone (60.16%, 74/123), and cefepime (60.16%, 74/123) followed by carbapenems (60.16%, 74/123) and quinolones, ciprofloxacin (60.16%, 74/123) and. The lowest resistance rate was observed against tigecycline (1.62%, 2/123). In addition, 75 (60.97%) of the 123 isolates were multi-drug resistance (MDR), 17.07% (21/123) were resistant to four antimicrobial classes, 22.76% (28/123) were resistant to five antimicrobial classes, and 20.33% (25/123) were resistant to all six antimicrobial classes.

## Prediction of Antimicrobial Resistance Genes (ARGs)

The genome sequences of 123 clinical *A. baumannii* isolates were analyzed for ARGs. A total of 40 different ARGs encoding resistance towards six classes of antimicrobial drugs were found in *A. baumannii* genomes (Figure 2). The most prevalent genes were *bla*<sub>ADC-25</sub> (100%,123/123), encoding resistance to cephalosporins, followed by *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub> (60.16%, 74/123), harbored resistance to carbapenems; *sul2* (47.97%, 59/123) encoding resistance to sulfonamides; *tet*(B) (48.78%, 60/123) encoding resistance to tetracyclines and *aph* (3'')-Ib (47.97%,59/123), *aph* (6)-Id (47.97%,59/123) harbored resistance to aminoglycosides. Furthermore, the trend of ARGs in our hospital over the last three years revealed a significant increase in the proportion of aminoglycosides, and macrolide ARGs, such as *armA* from 21.05% to 49.02% and *mph*(E) from 19.30% to 31.37%. However, the ARGs for  $\beta$ -lactam and sulfonamides showed a decreasing trend over time. Compared with 2019, *bla*<sub>TEM-ID</sub> decreased from 45.61% to 36.36% in 2021, and *sul* decreased by about 10% (Figure S1).

## Multi-Locus Sequence Typing (MLST)

The MLST results of 123 clinical *A. baumannii* isolates revealed 36 STs according to the Pasteur scheme (Figure 3a). Among them, ST2 was the predominant ST, 74/123 (60.16%), followed by ST40, 8/123 (6.50%). The MLST of isolates showed that ST2 was the most common ST from respiratory tract origin, 64/106 (60.38%), followed by ST40, 5.66% (6/106). All the clinical isolates from blood were ST2 while all isolates from bile belonged to ST40. The MLST of isolates from urine were identified as ST10, ST25, ST57, ST218, and ST2265. According to the years of isolations, 16 STs were isolated in 2019, 8STs were recovered in 2020, and in 2021 a total of 19 STs were isolated (Figure S2). The minimum spanning tree (MST) showed phylogenetic relationships of the sequence types (STs) using allele profiles of 123 clinical *A. baumannii* isolates. According to the MST, ST2 was primarily from ICU patients (82.89%,63/76), whereas ST40 was distributed across ICU patients (25%, 2/8), patients in internal medicine (37.5%, 3/8), and patients in surgery (37.5%, 3/8). Compared to the ICU (12 STs) and surgery departments (4STs), internal medicine (28 STs) has a more significant number of STs (Figure 3b).



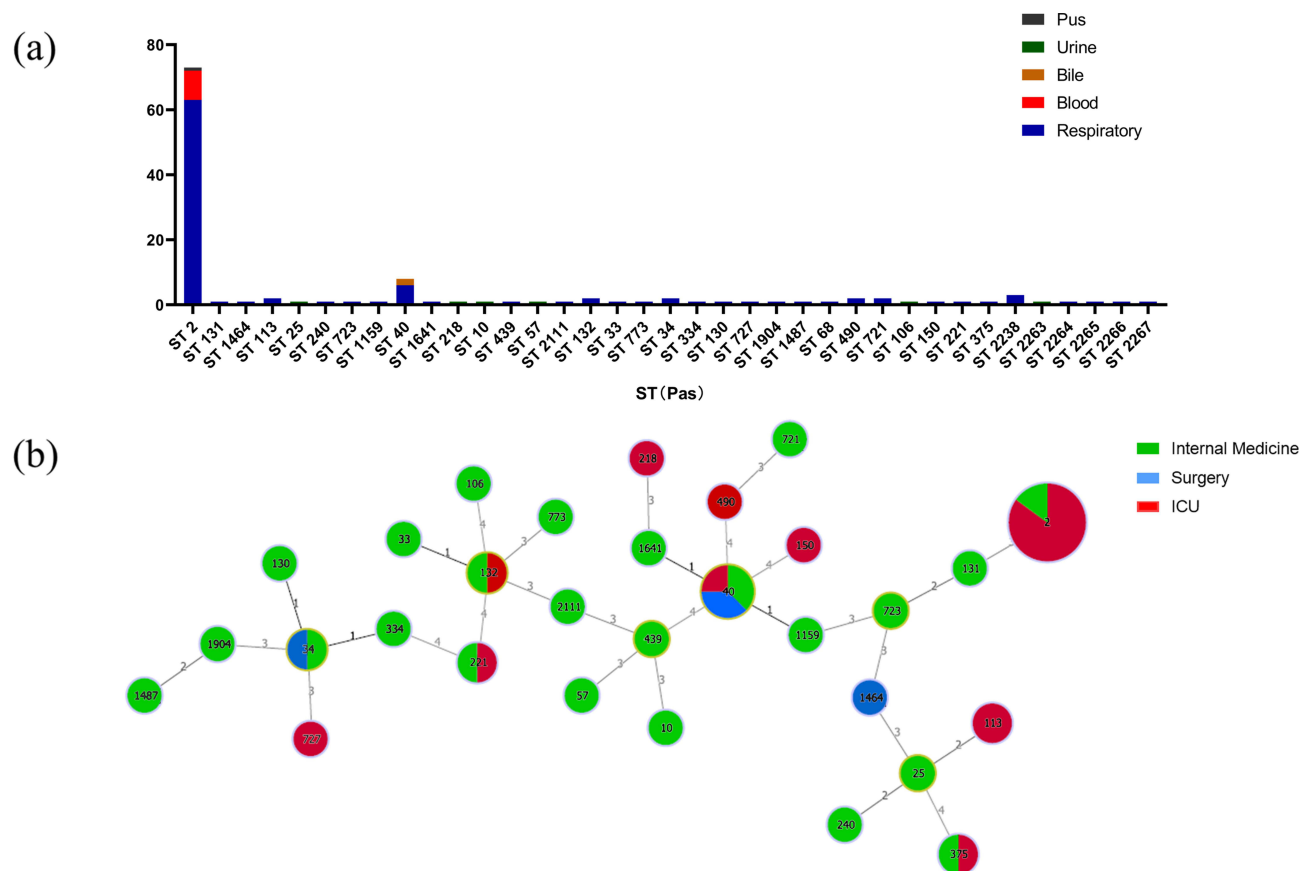
**Figure 2** The presence of antimicrobial resistance genes (ARGs) of 123 *Acinetobacter baumannii* clinical isolates.

**Notes:** The percentage (%) is calculated by the number of each ARGs /total number of isolates.

## Prediction of Virulence Factor Genes (VFGs) in *Acinetobacter baumannii* Genomes

The results of VFGs were predicted based on the virulence factor database (VFDB) presented in [Table S3](#). 123 clinical isolates of *A. baumannii* contained 7 major categories of VFGs, including adherence, biofilm formation, enzyme, immune evasion, iron uptake, regulation, and serum resistance. A total of 47 different VFGs belonging to 12 sub-categories were found. All isolates possessed genes encoding OmpA (outer membrane protein A), AdeFGH efflux pump, Phospholipase C, Phospholipase D, Acinetobactin, LPS (lipopolysaccharide), BfmRS (regulation of biofilm formation), and PbpG (penicillin-binding protein). 82 (66.67%), 86 (69.92%) to 97 (78.86%), 120 (97.56%) to 123 (100%), and 102 (82.93%) to 104 (84.55%) isolates possessed virulence factors including biofilm-associated proteins (*bap*), Csu fimbriae,





**Figure 3** Multi-locus sequence typing (MLST) and minimum spanning tree (MST) population analysis of 123 *Acinetobacter baumannii* clinical isolates from the different clinical departments.

**Notes:** (a) Genetic background with sequence types (STs) of 123 *A. baumannii* isolates from various clinical departments. (b) Minimum spanning tree (MST) based on STs profiles of 123 *A. baumannii* clinical isolates. Each circle represents an ST and the size of the circle is proportional to the number of isolates, and the color within the circle represents the section of the isolate. Links between circles are indicated according to the number of allelic mismatches between STs.

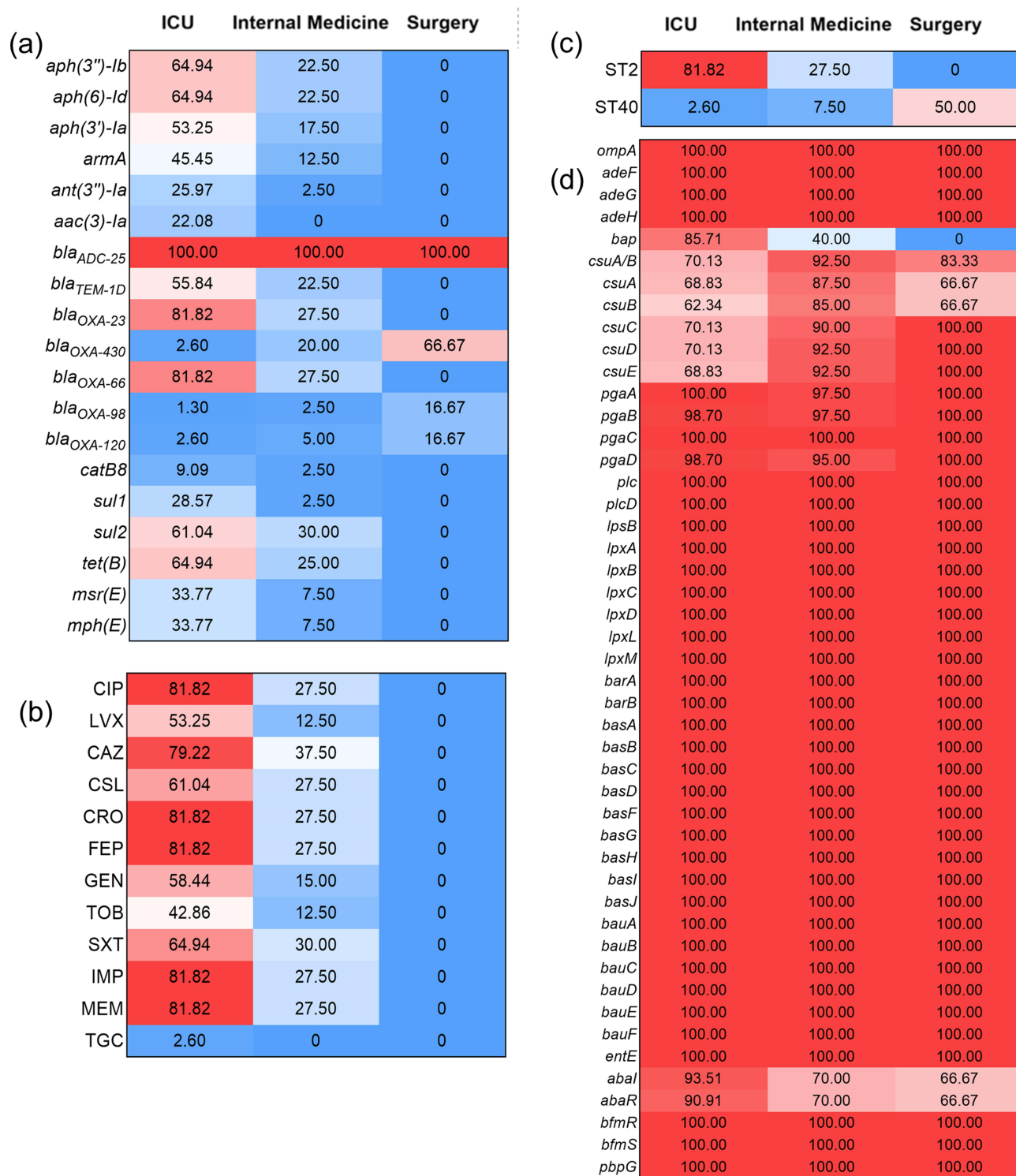
poly-N-acetylglucosamine (PANG) and quorum sensing, respectively. In addition, 56.76% (42/74) of ST2 isolates harbored all 7 major classes of VFGs.

## Differences in Antimicrobial Resistance Phenotypes, Genotypes, STs and VFGs in Clinical Departments

Among the 123 strains of *A. baumannii*, the resistance rate of ICU isolates was higher than that of the other two departments, especially ciprofloxacin, ceftriaxone, cefepime and carbapenems. In addition, ICU isolates also have the highest carrying rate of ARG, such as aminoglycosides (*aph* (3'')-Ib, *aph* (6)-Id), most cephalosporins (*bla*<sub>OXA-23</sub>, *bla*<sub>OXA-66</sub> and *bla*<sub>TEM-1D</sub>), sulfonamides (*sul2*), and tetracyclines (*tetB*). The ICU isolates were mainly ST2, while the ST40 strain mainly come from surgery. The isolates from three departments all carried a large number of VFGs. The *abaI/R* carrying rate of ICU isolates was high, but the *csu* pili of the isolates from internal medicine and surgery departments was higher than that of ICU isolates. There were significant differences in AMR phenotypes ( $P < 0.0001$ ), resistance genotypes ( $P = 0.0002$ ), and VFGs ( $P < 0.0001$ ) between isolates from different clinical departments (Figure 4).

## Correlation Analysis Between AMR Profiles, STs and VFGs

The correlation analysis showed that all observed correlation results were statistically significant ( $P < 0.05$ ) (Figure S3a–d). ARGs and phenotypic antibiotic resistance correlation analysis showed that carbapenems were most strongly correlated with  $\beta$ -lactamase resistance genes *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-66</sub>, *bla*<sub>TEM-1D</sub>, aminoglycoside genes *aph* (3'')-Ib, *aph* (6)-Id and tetracycline gene *tetB* (Figure S3a). STs and phenotypic antibiotic resistance correlation analysis demonstrated a strong



**Figure 4** Comparison of antimicrobial resistance phenotypes and genotypes, STs and VFGs between isolates from different clinical departments.

**Notes:** Based on (a) Antimicrobial resistance phenotypes, (b) Antimicrobial resistance genotypes, (c) STs and (d) VFGs. The numbers in cells correspond to the percentage (%).

correlation between ST2 strains and resistance to cephalosporins, carbapenems, quinolones, and aminoglycosides (Figure S3b). STs and ARGs correlation analysis illustrated that ST2 was strongly correlated with  $\beta$ -lactamase resistance genes *bla<sub>OXA-23</sub>* and *bla<sub>OXA-66</sub>*, the aminoglycoside genes *aph(3'')-Ib*, *aph(3')-Ia*, *aph(6)-Id*, the tetracycline gene *tet(B)*, and the sulfonamide gene *sul2* (Figure S3c). STs and VFGs correlation analysis displayed a strong correlation between ST2 and



*bap*, and some negative correlation with virulence factor *Csu* fimbriae; ST723 and *pgaA* showed absolute negative correlation (Figure S3d).

## Prediction of Insertion Sequence (ISs)

The results of insertion sequences of 123 strains of *A. baumannii* showed that 124 kinds of insertion sequences (ISs) were identified. We found that the most common ISs were IS*Aba22* (86.18%, 106/123) and IS*Aba26* (81.30%, 100/123). IS*Aba50*, IS*Aba64*, IS*Aba46* and IS*Aba16* were detected in 85 (69.10%), 83 (67.48%), 80 (65.04%) and 78 (63.41%) strains, respectively (Table S4). IS*Aba1* was detected in all CRAB isolates.

## Genetic Relationship and Phylogenomic Analysis of the Isolates

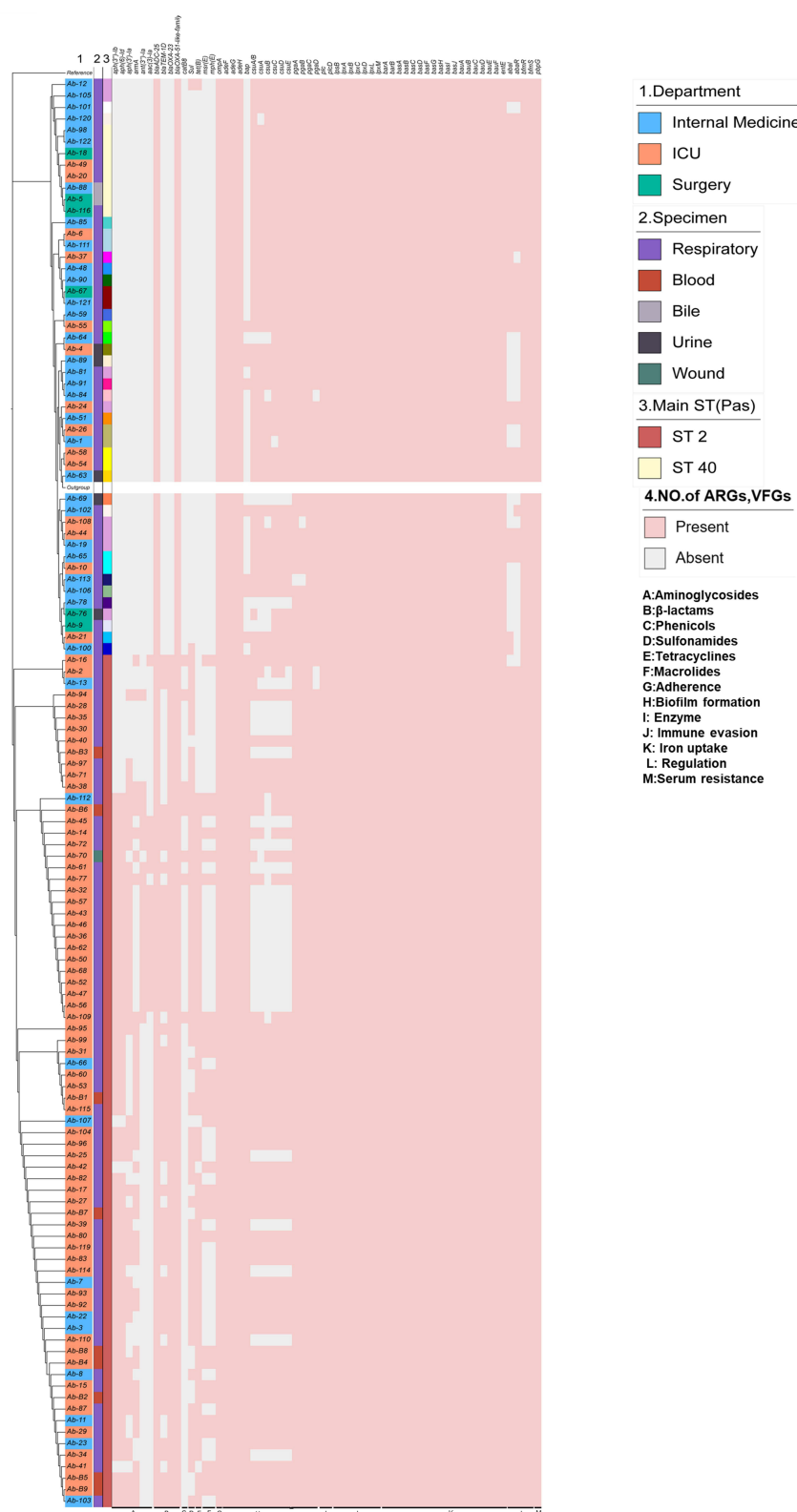
The maximum likelihood (ML) phylogenetic tree was constructed from the best model TVM+F+R3 of IQ tree v1.6.12 using 4744 SNPs, identified by core genome matching in Snippy v3.1. The phylogenetic tree (Figure 5) showed that the upper branch was dominated by strains from internal medicine “(blue)” and surgery “(green)”, with a large number of strains from the ICU “(orange)” in the lower branch. Phylogenomic analysis showed that most of the non-ST2 strains did not carry resistance genes such as *aph* (3’)-*Ib* and *aph* (6)-*Id*, and most strains showed the absence of chloramphenicol resistance genes, and respiratory isolates comprising of ST 40 were very similar to the bile isolates.

## Discussion

In this study, 75 of 123 patients with *Acinetobacter baumannii* had MDR-AB infections. 70% of the patients were between the ages of 62 and 89, with the majority being middle-aged or elderly individuals. Similar findings were observed in a previous study where *A. baumannii* infections were more prevalent in elder patients.<sup>42</sup> We found that most of the isolates recovered from the ICU department, and the infection site was predominantly identified in the respiratory tract, which may be due to their low immunity, and the use of ventilators for assisted ventilation and/or invasive surgery, which made a higher risk of nosocomial infection,<sup>43,44</sup> indicating that older patients in ICU were highly prone to *A. baumannii* infections. Our current findings were consistent with the global data.<sup>45</sup> In addition, our study found significant differences between isolates from ICU and other clinical departments in terms of AMR phenotypes, genetic determinants and virulence factor genes (VFGs). Many nosocomial infections were treatable with medicines; however, the increase in AMR rate has resulted in favoring the use of wide-spectrum antimicrobials in clinical practices, which in turn could lead to antimicrobial selection effects, conversely, this made it challenging to treat AB-infected patients.<sup>46,47</sup>

The results of phenotypic AMR profiling showed that the resistance rate of MDR-AB to cephalosporins and carbapenems was higher than aminoglycosides, fluoroquinolones, tigecycline, and cefoperazone-sulbactam sodium.<sup>48</sup> The analysis of clinical diagnosis and antibiotics used in the infected patients revealed that the most used antimicrobial drug in clinical practice was the enzyme-inhibitor complex (cefoperazone-sulbactam sodium or piperacillin-sulbactam), followed by the combined application of two or more antimicrobial drugs. For the anti-infective treatment of MDR-AB infection, enzyme-inhibitor complexes are preferred, and the combination of antimicrobial drugs could control the development of the disease as soon as possible.<sup>49–51</sup>

The results of ARGs analysis showed that a variety of resistance genes were identified in clinical *A. baumannii* isolates from hospitalized patients in different clinical departments. All isolates contained *bla*<sub>ADC-25</sub>, which is generally considered to be an important genetic determinant of cephalosporin resistance in *A. baumannii*.<sup>52</sup> Moreover,  $\beta$ -lactamase resistance gene *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub>-like variant (*bla*<sub>OXA-66</sub>) were more prevalent in the 123 genomes and associated with resistance to all  $\beta$ -lactam based compounds.<sup>53</sup> A few isolates carried *bla*<sub>TEM-1D</sub>, which encodes a class A  $\beta$ -lactamase. In Carbapenem-resistant *Acinetobacter baumannii* (CRAB) from different regions of the world, it was found that the isolates carrying *bla*<sub>TEM-1D</sub> were sensitive to carbapenems,<sup>54</sup> cephalosporins and enzyme-inhibitor complex, but showed resistance to other  $\beta$ -lactams, aminoglycosides and quinolones. In addition to  $\beta$ -lactam resistance genes, these isolates contained genes that likely conferred resistance to aminoglycoside, tetracycline, chloramphenicol, sulfonamide, and macrolide. In addition, the three-year trend of ARGs showed that the proportion of aminoglycosides, macrolides, and tetracycline ARGs increased over time. The increase in bacterial ARGs and phenotypic resistance indicated that hospitals had to take strict measures to control the use of relevant antimicrobial drugs.



**Figure 5** Phylogenetic analysis and heat map of the detection of ARGs and VFGs among the 123 *Acinetobacter baumannii* clinical isolates.

**Notes:** *Acinetobacter baumannii* isolates were used to construct a maximum-likelihood tree with 100 bootstraps. Single nucleotide polymorphisms SNPs-based phylogenetic tree of *A. baumannii* genomes. 4744 SNPs were identified on the basis of whole-genome alignment of 123 sequenced. ATCC19606 was used as the reference strain and *Acinetobacter lwoffii* was used as an out-group strain. The upper branch was dominated by strains from internal medicine "(blue)" and surgery "(green)", with a large number of strains from the ICU "(orange)" in the lower branch. The tree scale represents the genetic distance between the isolates used to construct the tree.

ST2 was the most dominant ST, which belonged to “international clone 2 (IC2)”.<sup>55</sup> The resistance rate of ST2 isolates to antibacterial drugs and carrying rate of ARGs was relatively far higher than other STs, especially the resistance rate to  $\beta$ -lactam drugs. These results were consistent with the previous findings.<sup>18</sup> The correlation analysis between STs and antibiotic resistance phenotypes and ARGs showed that ST2 isolate showed a strong correlation with the resistance genes *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-66</sub>, cephalosporins and carbapenems. In this study, ST2 was mainly identified from ICU isolates, while ST40 was distributed in ICU, internal medicine and surgery department, and the number of STs from internal medicine was much higher than the other two clinical departments. Notably, all isolates from blood specimens belonged to ST2. Although STs were complicated, the major STs did not change over three years. ST2 was the most prevalent and mainly from ICU, indicating that the isolates recovered from ICU were genetically homogenous and had the ability for horizontal transmission of resistance genes.

Nevertheless, *A. baumannii* was once thought to be a low-virulent pathogen, but in recent years, researchers have revealed the presence of highly virulent *A. baumannii*.<sup>56,57</sup> In this study, we found many virulence factor genes, including adherence, biofilm formation, enzyme, immune evasion, iron uptake, regulation, and serum resistance. These results were consistent with the previous study.<sup>13</sup> In our study, all the isolates had BfmRS indicating that these isolates were motile. It was well known that *A. baumannii* exhibited loss of BfmRS<sup>58</sup> or AbaI/R quorum sensing system<sup>59</sup> leading to a significant reduction in motility. More than half of ST2 isolates contained all seven major classes of VFGs in this study, implying that ST2 isolates carried more VFGs and were more viable in the host environment. This might be the reason that ST2 had become a prevalent clone worldwide.

We predicted the insertion sequence (IS) of 123 *A. baumannii* strains, and among the 124 ISs identified in our study, we found IS*Aba22* to be the most prevalent IS, followed by IS*Aba26*, IS*Aba50*, IS*Ab64a*, and IS*Aba46*. In addition to functioning as a promoter for the expression of many ARGs, IS usually provided a copy-and-paste mechanism of transposition.<sup>60</sup> Based on our findings, IS*Aba1* was identified in all *bla*<sub>OXA-23</sub> positive CRAB isolates. Many previous reports suggested that IS*Aba1* was usually located upstream of *bla*<sub>OXA-23</sub>-like genes and that it provided a powerful promoter that drive the expression of *bla*<sub>OXA23-like</sub> genes.<sup>61</sup>

Our phylogenetic analysis showed that the distribution of isolates from respiratory specimens was relatively concentrated, indicating that *A. baumannii* can be easily transmitted through the respiratory tract route. The isolates from the respiratory tract were similar to those from bile, indicating that bile-derived *A. baumannii* might have come from respiratory infection. Moreover, the blood isolates were closely linked with respiratory isolates, which suggested that the bloodstream infection of the clinical *A. baumannii* isolates was more likely to be from a respiratory infection.<sup>13,62</sup> The phylogenetic distribution of our blood-infected isolates was dispersed, suggesting a distant relationship and perhaps not a single route of entry into the bloodstream from the respiratory tract or other sites. Treating patients in different clinical departments could be another factor that causes the risk of cross-sectoral transmission of *A. baumannii*. Hence, it was necessary to enhance hygienic and sanitizing measures as well as detection capabilities for *A. baumannii*. Additional recording of the traceability of individual patient could help to limit the persistence and dissemination of *A. baumannii* in and between clinical departments.

## Conclusion

The present study concluded that 123 clinical isolates of *A. baumannii* showed a high rate of antimicrobial resistance pattern. Especially, isolates from the ICU department were more resistant to antimicrobial drugs and might be more virulent. Most of the *A. baumannii* were found in ICU respiratory specimens, which indicated that it is challenging to treat ICU-acquired pneumonia. This was most common in older people, who speeded long periods of time on artificial ventilation; ST2 was found to be the prevalent sequence type in our hospital. *A. baumannii* isolates revealed high rate of virulence factor carriage, which indicated the pathogenicity of various prevalent clones.

## Data Sharing Statement

The datasets generated for this study can be found in the NCBI Bioproject with the accession number PRJNA873189.

## Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of Hangzhou First People's Hospital [Approval numbers 2019-020-01]. Waiving of informed consent was given due to the retrospective, non-interventional study design. All patient's data were collected anonymously and ensured about the confidentiality of their information.

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## Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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