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Epidemiology and molecular typing of multidrug resistant *Acinetobacter baumannii* burn wound isolates from six Chinese provinces

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Acinetobacter baumannii **(*A. baumannii*) is a pathogen that opportunistically infects patients in healthcare settings and poses a significant threat to human health due to its widespread resistance to antimicrobial agents. The temporal and regional distribution of *A. baumannii* in China is continually evolving, necessitating comprehensive surveillance to examine the prevalence, molecular characteristics, and clonal relationships of antimicrobial resistance determinants in burn wound *A. baumannii* isolates collected from six major Chinese provinces during 2016–2020. To address this need, we analyzed a total of 415 distinct *A. baumannii* isolates that were obtained from burn patients. Antimicrobial susceptibility testing was performed using the Kirby Bauer disk diffusion method, while genetic relatedness of the 415 isolates was assessed by utilizing multilocus sequence typing (MLST) and eBURST analysis. Additionally, the housekeeping genes and a comprehensive panel of drug resistance genes, including β-lactamases, membrane permeability factors, and efflux pump systems, were detected by multiplex PCR to elucidate their resistance mechanisms. Our analysis revealed that among the 415 isolates, 384 (92.5%) were classified as multidrug-resistant (MDR), with regional rates ranging from 88.2 to 96.0%. Additionally, 38 (9.2%) isolates were classified as extensively drug-resistant (XDR). All strains showed resistance to multiple antibiotics, except for lower resistance to Tigecycline (TGC) and Cefoperazone/sulbactam (CSL), suggesting their potential therapeutic utility. By MLST, all isolates represented 122 identified STs (sequence types). eBURST analysis identified the 415 isolates into 17 clonal complexes (CCs) in which CC1660 and CC1417 were considered large CCs with at least 100 isolates in each CC. Importantly, our investigation identified a notable epidemiological shift: CC1417 and CC1660 instead of CC92 have become the novel predominant strains in burn wound isolates of these Chinese provinces, and the predominant strain varied in separate regions. Consistently, our investigation identified ST1417, ST1660, and ST1145 instead of ST208 as predominant sequence types, indicating a notable shift in regional epidemiological patterns for burn wound *A. baumannii* isolates. In addition, the carrying rates of multiple antibiotic-resistant genes maintain a high level, indicating the evolution of strain genes and the severe drug resistance situation in burn wound isolates of these Chinese provinces. As a result, epidemiological surveillance and genetic evolution analysis of *A. baumannii* is of great significance to provide more strategies for the prevention of nosocomial infection and clinical treatment.**

Keywords *A. baumannii*, Multidrug-Resistant, Resistance genes, Sequence Types, Molecular epidemiology,

Genetic evolution

Abbreviations

A. baumannii	*Acinetobacter baumannii*
CAZ	Ceftazidime
CC	Clonal Complex
CIP	Ciprofloxacin
CSL	Cefoperazone/sulbactam
CTX	Cefotaxime
GEN	Gentamicin
ICU	Intensive Care Unit
IPM	Imipenem
LB	Lysogeny broth
MDR	Multidrug-Resistant
MEM	Meropenem
MIC	Minimum Inhibitory Concentration
min	Minutes
MLST	Multilocus sequence typing
PCR	Polymerase Chain Reaction
PIP	Piperacillin
PubMLST	Public databases for Molecular Typing
ST	Sequence Type
SXT	Trimethoprim/sulfamethoxazole
TGC	Tigecycline

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Acinetobacter baumannii (*A. baumannii*), a strictly aerobic opportunistic pathogen, has emerged as a formidable threat for burn patients[1,2]. In burn patients, *A. baumannii* demonstrates high nosocomial infection rates and frequently co-infects with other pathogens, resulting in difficult-to-treat infections associated with prolonged hospitalization and increased mortality[3,4]. Additionally, a significant public health issue regarding *A. baumannii* infection is detecting an increasing resistance rate to multiple antibiotics[5]. With the inappropriate use of antibiotics, the rising incidence of antimicrobial resistance has become an alarming phenomenon in clinical work[6], as it limits the therapeutic efficacy of antibiotics. Therefore, continuous epidemiological monitoring and infection analysis are of great significance for the study of treatment and control strategies for *A. baumannii* infection in burn patients[7]. The resistance mechanisms of *A. baumannii* include β-lactamases, modifications of target sites, permeability defects, efflux pumps, and aminoglycoside-modifying enzymes[8]. Of them, β-lactamase is the primary mechanism[9]. β-lactamase enzymes hydrolyze the β-lactam ring in antibiotics, resulting in antibiotic inactivation and bacterial resistance[10]. In addition, bacteria possessing efflux pump genes can expel antibiotics, which is associated with many different kinds of drug resistance, especially imipenem[7,11]. Previously, carbapenems served as the cornerstone of *A. baumannii* treatment, owing to their high efficiency and low toxicity[12]. However, recent surveillance data indicates an alarming increase in carbapenem-resistant isolates, particularly in the ICU ward. Imipenem (IPM) and meropenem (MEM) were the two most effective carbapenem antibiotics, but studies showed their drug-resistance situation was tricky[13]. A 2021 report collected 94 isolates from 2016 to 2019, of which 88 (93.6%) were resistant to imipenem or meropenem, with resistance rates showing a consistent upward trajectory[14]. The exceptional genomic plasticity of *A. baumannii* facilitates horizontal gene transfer of these resistance determinants, enabling rapid acquisition of novel resistance mechanisms[15].

Multilocus sequence typing can exactly show the characteristics of bacteria through housekeeping gene fragments[16], and an ST identifier is composed of seven housekeeping gene loci. Its remarkable success in global epidemiological investigation makes it the most popular typing method[16,17]. Studies have shown that the molecular type distribution of *A. baumannii* is geographically related[18]. In China, the prevalence of CC92 clones is widespread across most provinces, indicating the significant spread of this particular clone of *A. baumannii*[19].

Extensive research has been conducted to investigate the molecular epidemiology of this pathogen[20,21]. However, the epidemiology of *A. baumannii* in certain regions may offer new insights into its behavior and spread in the coming years. Further studies in these regions could contribute to our understanding of the disease and inform future strategies for its control and prevention.

The emergence and spread of multidrug-resistant (MDR) *A. baumannii* pose a significant threat to public health worldwide. In recent years, the epidemiology and resistance patterns of this bacterium have been extensively studied, with notable research conducted in different regions, including Asia. While previous studies, such as the ones by Wohlfarth et al.[22] in Germany and Nodari et al.[23] in South America, have shed light on the epidemiological landscape of MDR *A. baumannii*, the need to incorporate the latest findings from Asia, particularly China, remains pivotal. Recent research by Mo et al.[24] unveiled the alarming presence of blaNDM-1 harboring MDR *Acinetobacter bereziniae* isolates within South China's hospital settings, emphasizing the

pressing need for comprehensive surveillance and intervention strategies in the region. Furthermore, extensive surveillance studies in China have documented the widespread distribution of OXA-type carbapenemases, with blaOXA-23 being the predominant resistance mechanism carried within Tn2007 transposons across different provinces[1]. Additionally, large-scale genomic analyses have revealed that carbapenem-resistant *A. baumannii* (CRAB) shows an isolation rate of approximately 70% in Chinese hospitals, with these strains showing consistent susceptibility only to polymyxins and tigecycline[1].

Given these pressing concerns and the unique vulnerability of burn patients to *A. baumannii* infections[2], this study seeks to explore the contemporary landscape of *A. baumannii* burn wound isolates, particularly emphasizing the latest regional data from six major Chinese provinces. Molecular epidemiological studies have demonstrated that *A. baumannii* exhibits substantial regional heterogeneity due to multiple interconnected factors including geographical isolation, distinct transmission pathways, antibiotic selection pressures, and continuous bacterial evolution[25]. These factors collectively contribute to region-specific dominant clonal types and resistance patterns across different healthcare settings. The epidemiology analysis of *A. baumannii* can help demonstrate the current clinical situation of ST typing, resistance characteristics, regional distribution differences, and genotype characteristics for further precision medicine treatments.

Material and methods

This retrospective study was carried out under the approved guidelines of the Ethics Committee of Xinqiao Hospital and in accordance with the Declaration of Helsinki. No written informed consent was required as we received anonymized isolate samples with all the personal information removed.

Clinical isolate collections of *A. baumannii*

Burn wound isolates of *A. baumannii* were collected from burn department of central hospital in 6 Chinese provinces between 2016 and 2020, through their active surveillance system, including Jilin (123/415), Guangdong (25/415), Henan (112/415), Shaanxi (88/415), Jiangsu (17/415), and Chongqing (50/415) (detailed information in Fig. 1). These provinces represent different geographical regions of China (north eastern, south western, eastern and south eastern), which together contain over 93% of China's population according to the population distribution pattern defined by the Hu Huanyong Line[26]. Isolates were identified using standard microbiological methods including colony morphology, Gram staining, and biochemical tests[27]. Species identification was confirmed using MALDI-TOF mass spectrometry or 16S rRNA gene sequencing when necessary. Genomic DNA was extracted from overnight cultures using the TIANamp Bacteria DNA Kit (TIANGEN, Beijing), with quality verified by A260/A280 ratios (1.8–2.0) and 16S rRNA amplification[28]. The strains were stored at − 80 °C in glycerol broth for long-term preservation. For experimental procedures, isolates were retrieved from frozen stocks and cultured overnight in LB medium (Beyondtime, China) at 37 °C with shaking at 180 rpm for further studies. Resistance phenotype classification was performed according to standardized criteria. Multidrug-resistant (MDR) isolates were defined as those showing resistance to ≥ 3 antimicrobial categories with inhibition zone diameters ≤ 14 mm. Extensively drug-resistant (XDR) isolates were defined as those resistant to all tested antibiotics except 1–2 categories, determined by standard disk diffusion breakpoints.

Multilocus sequence typing

The molecular subtypes of all *A. baumannii* isolates were subjected to MLST by PCR amplification and sequencing of seven housekeeping genes (*gltA*, *gdhB*, *gyrB*, *recA*, *gpi*, *cpn60*, and *rpoD*) as described by the Oxford scheme introduced by Bartual[29,30]. To carry out PCR amplification, one microliter of DNA was added to 20 µL of the reaction mixture, which contains 10 µMol/L of each primer and 10 µL of 2xTaq premix (TIANGEN, Beijing). Then PCR was performed, and 5% DMSO (Sangon Biotech, Shanghai) should be added to the reaction if necessary. The reaction products were further separated and detected on an ABI PRISM genetic analyzer 3100 (Applied Biosystems, Waltham, MA). An identifier of the ST gene was constructed by combining 7 loci, and the sequencing results of each gene were compared with the known sequences in the PubMLST database (https://pubmlst.org/). The primers used in updated Oxford MLST scheme are listed in Table S1.

eBURST and phylogenetic analysis

Following MLST analysis, goeBURST (http://www.phyloviz.net/goeburst/) was used for data grouping analysis[31]. All reported MLST data were queried from the PubMLST database (https://pubmlst.org/). Clonal complexes (CCs) were first assigned using the classical MLST definition, in which STs sharing 6 out of 7 housekeeping alleles were grouped into the same CC. For each ST detected in this study, the complete allelic profiles deposited in PubMLST were retrieved and used as the reference for CC determination.

Besides, goeBURST was then applied to construct the single-locus-variant (SLV) network. Unlike the 6/7-allele rule used for CC classification, the SLV-based goeBURST network links STs differing at only one allele, thereby illustrating evolutionary connections among STs. Thus, CC assignment and SLV network construction were complementary but relied on different clustering principles. After the multisequence alignments with ClustalX2 (http://www.clustal.org/clustal2/)[32], an independent maximum-likelihood phylogenetic tree was generated using PhyML (http://www.atgc-montpellier.fr/phyml/)[33]. This phylogenetic analysis was performed to validate whether the ML-based evolutionary relationships were consistent with the population structure inferred from MLST and goeBURST.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed by the Kirby Bauer disk diffusion method for nine antibiotics[34], including sulfamethoxazole (SXT), Ciprofloxacin (CIP), Gentamicin (GEN), Ceftazidime (CAZ), Cefoperazone/sulbactam (CSL), Cefotaxime (CTX), Imipenem (IPM), Piperacillin (PIP) and Tigecycline (TGC). The selection

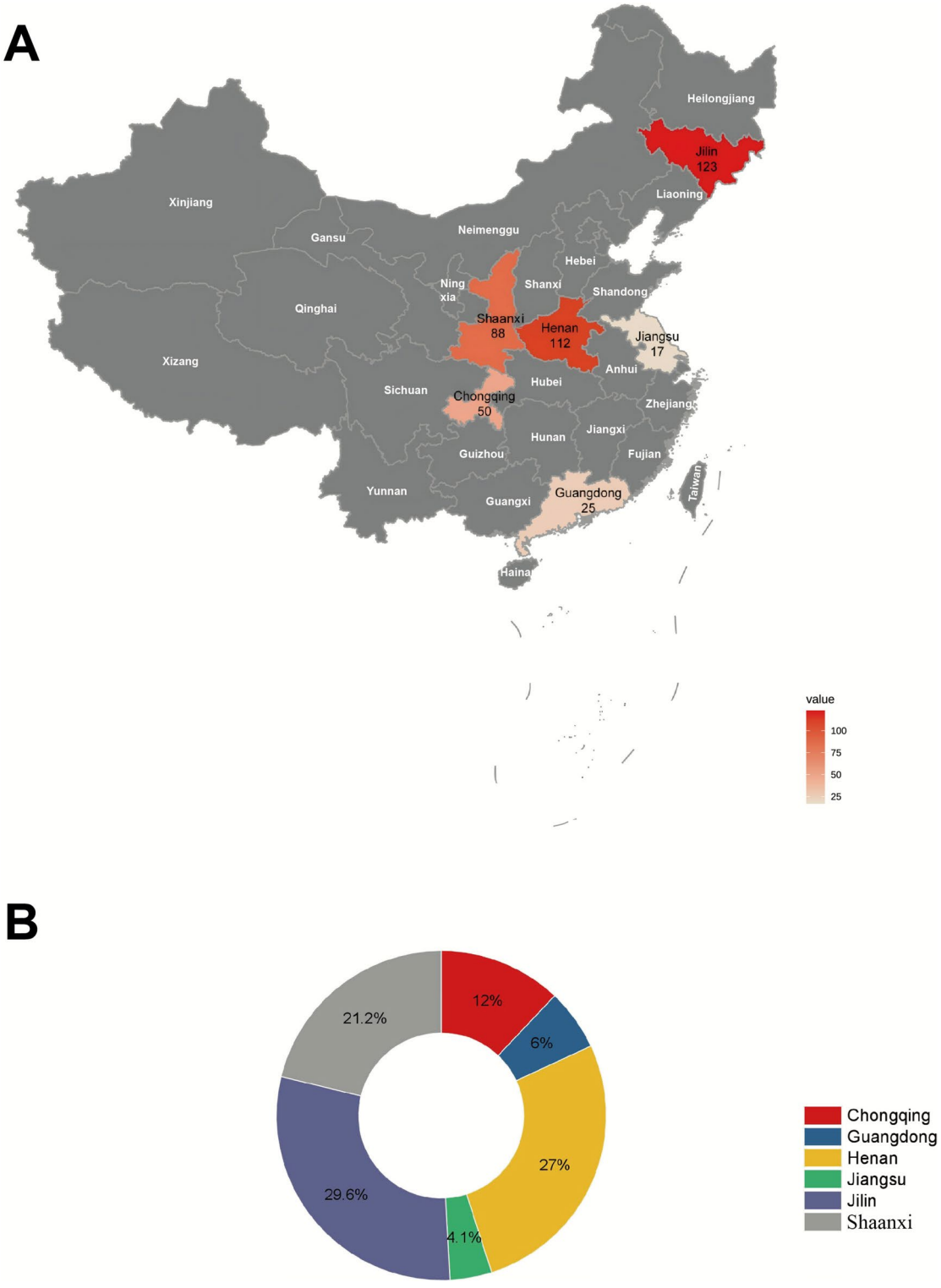

Fig. 1. Geographical distribution of all *A. baumannii* strains. (**A**) The highlighted area on the map is the origin of 415 quarantine points. Including Chongqing, Guangdong, Jilin, Henan, Shaanxi, and Jiangsu. (**B**) Pie chart distribution of 415 isolates in each province.

of these nine antibiotics was based on: (1) current evidence-based therapeutic approaches for multidrug-resistant *Acinetobacter baumannii* infections, as outlined in international treatment guidelines[35]; (2) recommendations from the Chinese guidelines for carbapenem-resistant Gram-negative bacilli infections[36]; and (3) clinical relevance, including both conventional first-line agents and last-resort antibiotics commonly used in Chinese healthcare settings. According to the Clinical and Laboratory Standards Institute guidelines (CLSI 2017,

M100-S27), the drug resistance of bacteria can be judged by comparing the diameter and breakpoint of the zone diameter[37]. The MIC of cefoperazone/sulbactam and tigecycline refers to the opinions of Sader HS et al.[38] and Jones et al.[39].

PCR-based screening for antimicrobial-resistant genes

Genomic DNA was extracted from overnight cultures using the TIANamp Bacteria DNA Kit (TIANGEN, Beijing), with quality verified by A260/A280 ratios (1.8–2.0) and 16S rRNA amplification. The 21 resistance genes were divided into 3 groups and detected by multiplex PCR[40]. Group1 included the β-Lactamases which can be divided into four categories: class A with *blaGES*, *blaKPC*, *blaPER*, *blaCTX*, class B with bla_{IMP}, bla_{VIM}, bla_{SIM}, bla_{NDM}, class C with bla_{AMP}, and class D with bla_{OXA}. Group 2 included the permeability defects with *carO, ompW*, and *omp33-66*. Group 3 included the efflux pumps with *Ade*. The primers used in screening these resistance genes are listed in Table S2. The conditions for PCR reactions were as follows : pre-denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 15 min.

Results

MLST and population structure analysis

Four hundred and fifteen isolates were subjected to typing using the updated Oxford Bartual scheme of MLST. The isolates were divided into 122 STs, reflecting the high clonal diversity of *A. baumannii* (Table 1). The study found that the six most prevalent STs of *A. baumannii* isolates included ST1417 (45/415, 10.8%), ST1660 (36/415, 8.7%), ST1145 (32/415, 7.7%), ST1661 (26/415, 6.3%), ST1646 (22/415, 5.3%), and ST1679 (20/415, 4.8%), collectively representing 43.6% of all isolates (Tables 1 and S3). Notably, we observed distinct regional patterns in ST distribution (Fig. 2). ST1417 dominated in Henan (18/112, 16.1%) and Shaanxi (15/88, 17.0%) provinces, while ST1679 was also prevalent in Henan (16/112, 14.3%) (Fig. 2). Jilin Province showed high prevalence of ST1660 (11/123, 8.9%) and ST1145 (15/123, 12.2%) (Fig. 2). In Shaanxi, ST1660 (14/88, 15.9%) emerged as another major strain alongside ST1417 (Fig. 2). Jiangsu exhibited co-dominance of ST1660 (5/17, 29.4%) and ST1661 (5/17, 29.4%), while Chongqing showed a unique predominance of ST368 (14/50, 28.0%) (Fig. 2).

eBURST analysis

Using the classical MLST definition (STs sharing 6 of the 7 alleles), the 122 STs identified in this study were assigned into 17 clonal complexes based on their full PubMLST allelic profiles. Consistent with established nomenclature, several STs detected in this study (e.g., ST195, ST208, and ST368) belonged to the literature-defined CC92 lineage, even though ST92 itself was not detected among our isolates. Geographical comparison of the major CCs revealed distinct regional patterns. CC1417 was widely distributed across the sampled provinces (Fig. 3A), whereas CC1660 predominated in four provinces (Fig. 3B). CC92 exhibited strong regional specificity, with most isolates concentrated in Chongqing (Fig. 3C). Additionally, because ST1145 was one of the more common sequence types in our dataset (32/415, 7.7%), we examined its geographical distribution (Fig. 3D). ST1145 showed higher prevalence in Jilin Province (15/123, 12.2%) and was absent in Guangdong. This pattern indicates unequal distribution across provinces.

To further explore the evolutionary relationships among STs, the goeBURST algorithm was applied to construct a single-locus-variant (SLV) network. As goeBURST relies on one-allele differences rather than the 6/7 shared-allele criterion used for CC assignment, it provides complementary information by resolving evolutionary relationships. The SLV network identified three major founder-centered clusters: CC1417 (centered on ST1646), CC1660 (centered on ST1665), and the CC92 lineage (centered on ST195), illustrating the diversification pathways and genetic structure within A. baumannii populations (Fig. 3E). Finally, to independently validate the evolutionary structure inferred from MLST and the goeBURST network, a phylogenetic tree was generated using PhyML. The phylogeny showed clustering patterns consistent with the CC assignments and the SLV-based network, with no conflicting topologies, supporting the robustness of the inferred population structure.

Antimicrobial susceptibility profiles

To evaluate antimicrobial resistance profiles, we tested the isolates against nine clinically relevant antibiotics. The results revealed consistently high resistance rates across most antibiotics: ciprofloxacin (CIP, 91.57%, 380/415), gentamicin (GEN, 88.92%, 369/415), piperacillin (PIP, 90.84%, 377/415), trimethoprim/sulfamethoxazole (SXT, 82.65%, 343/415), ceftazidime (CAZ, 91.08%, 378/415), cefotaxime (CTX, 91.57%, 380/415), and imipenem (IPM, 90.60%, 376/415) (Table 2). Notably, lower resistance rates were observed for tigecycline (TGC, 37.35%, 155/415) and cefoperazone/sulbactam (CSL, 69.40%, 288/415) (Table 2). Compared with the isolates from Jiangsu, strains from Chongqing, Guangdong, Henan, Shaanxi, and Jilin showed higher resistance to most antibiotics (Table 2). Of particular clinical significance was the finding that four provinces exhibited IPM resistance rate exceeding 90% (Table 2). Furthermore, it has been observed that Jilin province exhibited the highest prevalence of TGC resistance, with a rate of 76%, which was three to nine times that of the other five regions (Table 2). The bacterial isolates originating from Jilin province have demonstrated a high level of resistance towards various antibiotics with a 100% resistance rate towards CIP and CAZ, and above 88% towards other antibiotics. Based on the comprehensive resistance patterns observed, 384 isolates (92.5%) met MDR criteria (Table 2). Guangdong showed the highest MDR rate (96.0%), while Jiangsu demonstrated the lowest (88.2%). Furthermore, 38 isolates (9.2%) exhibited XDR phenotypes. Guangdong had the highest XDR prevalence (12.0%), followed by Shaanxi (11.7%). Chongqing showed the lowest XDR rate (4.0%) (Table 2). Furthermore, our investigation has revealed the differences in antimicrobial resistance rates among major *A. baumannii* lineages. While the remaining five clones displayed a generally elevated drug resistance, the resistance rate of ST1679 was notably elevated in

ST	Total_Numbers	Chongqing	Guangdong	Henan	Jiangsu	Jilin	Shaanxi
*1417	45	1	3	18	1	7	15
*1660	36	0	0	6	5	11	14
*1145	32	2	0	5	3	15	7
*1661	26	0	0	3	5	9	9
*1646	22	8	5	0	0	8	1
*1679	20	0	0	16	0	3	1
368	14	14	0	0	0	0	0
1670	13	0	0	13	0	0	0
1709	12	0	0	1	0	9	2
1665	11	0	0	10	0	1	0
1699	10	0	0	1	0	2	7
208	8	3	4	0	0	1	0
1658	7	0	1	2	0	0	4
1721	6	0	0	0	0	6	0
1766	6	0	0	5	0	0	1
195	5	4	1	0	0	0	0
1144	5	1	0	4	0	0	0
1659	4	0	4	0	0	0	0
1811	4	0	0	2	0	2	0
1669	3	0	0	3	0	0	0
1681	3	0	0	1	0	0	2
1711	3	0	0	0	0	1	2
1712	3	0	0	0	0	3	0
1732	3	0	0	0	0	3	0
1741	3	0	0	0	0	3	0
1742	3	0	0	0	0	3	0
136	2	0	2	0	0	0	0
1408	2	2	0	0	0	0	0
1650	2	2	0	0	0	0	0
1656	2	0	1	1	0	0	0
1666	2	0	0	2	0	0	0
1695	2	0	0	1	0	0	1
1720	2	0	0	0	0	1	1
1725	2	0	0	0	0	2	0
1727	2	0	0	0	1	1	0
1731	2	0	0	0	0	2	0
1750	2	0	0	0	0	0	2
1785	2	0	0	0	0	0	2
75	1	1	0	0	0	0	0
381	1	1	0	0	0	0	0
829	1	1	0	0	0	0	0
931	1	1	0	0	0	0	0
1215	1	0	0	0	0	1	0
1247	1	0	1	0	0	0	0
1645	1	1	0	0	0	0	0
1647	1	1	0	0	0	0	0
1648	1	1	0	0	0	0	0
1649	1	1	0	0	0	0	0
1651	1	1	0	0	0	0	0
1652	1	1	0	0	0	0	0
1653	1	1	0	0	0	0	0
1654	1	1	0	0	0	0	0
1655	1	1	0	0	0	0	0
1657	1	0	1	0	0	0	0
1662	1	0	1	0	0	0	0
1663	1	0	1	0	0	0	0

Continued

ST	Total_Numbers	Chongqing	Guangdong	Henan	Jiangsu	Jilin	Shaanxi
1664	1	0	0	1	0	0	0
1668	1	0	0	1	0	0	0
1672	1	0	0	1	0	0	0
1673	1	0	0	1	0	0	0
1674	1	0	0	1	0	0	0
1676	1	0	0	1	0	0	0
1680	1	0	0	1	0	0	0
1690	1	0	0	1	0	0	0
1696	1	0	0	1	0	0	0
1698	1	0	0	1	0	0	0
1700	1	0	0	1	0	0	0
1702	1	0	0	1	0	0	0
1706	1	0	0	0	0	1	0
1707	1	0	0	0	0	1	0
1708	1	0	0	0	0	1	0
1710	1	0	0	0	0	1	0
1713	1	0	0	0	0	1	0
1716	1	0	0	0	0	1	0
1718	1	0	0	0	0	1	0
1722	1	0	0	0	0	1	0
1723	1	0	0	0	0	1	0
1726	1	0	0	0	0	1	0
1730	1	0	0	0	0	1	0
1735	1	0	0	0	0	1	0
1737	1	0	0	0	0	1	0
1738	1	0	0	0	0	1	0
1743	1	0	0	0	0	1	0
1744	1	0	0	0	0	1	0
1745	1	0	0	0	0	0	1
1746	1	0	0	0	0	0	1
1747	1	0	0	0	0	1	0
1748	1	0	0	0	0	0	1
1749	1	0	0	0	0	0	1
1751	1	0	0	0	0	0	1
1753	1	0	0	0	0	0	1
1754	1	0	0	0	0	1	0
1755	1	0	0	0	0	0	1
1756	1	0	0	0	0	1	0
1758	1	0	0	0	0	0	1
1762	1	0	0	0	0	1	0
1763	1	0	0	0	0	0	1
1765	1	0	0	0	0	0	1
1767	1	0	0	0	0	0	1
1768	1	0	0	0	0	1	0
1769	1	0	0	0	0	0	1
1770	1	0	0	0	0	0	1
1771	1	0	0	0	0	0	1
1781	1	0	0	0	0	0	1
1783	1	0	0	0	0	0	1
1784	1	0	0	0	0	0	1
1786	1	0	0	1	0	0	0
1787	1	0	0	1	0	0	0
1797	1	0	0	1	0	0	0
1799	1	0	0	1	0	0	0
1802	1	0	0	1	0	0	0
1810	1	0	0	1	0	0	0
Continued							

ST	Total_Numbers	Chongqing	Guangdong	Henan	Jiangsu	Jilin	Shaanxi
1817	1	0	0	0	0	1	0
1822	1	0	0	0	0	1	0
1823	1	0	0	0	0	1	0
1824	1	0	0	0	0	1	0
1826	1	0	0	0	0	1	0
1827	1	0	0	0	0	1	0
1828	1	0	0	0	0	1	0
1829	1	0	0	0	0	1	0
1831	1	0	0	0	1	0	0
1832	1	0	0	0	1	0	0

Table 1. STs (sequence types) found in this study. *Indicates top 6 ST strains by numbers.

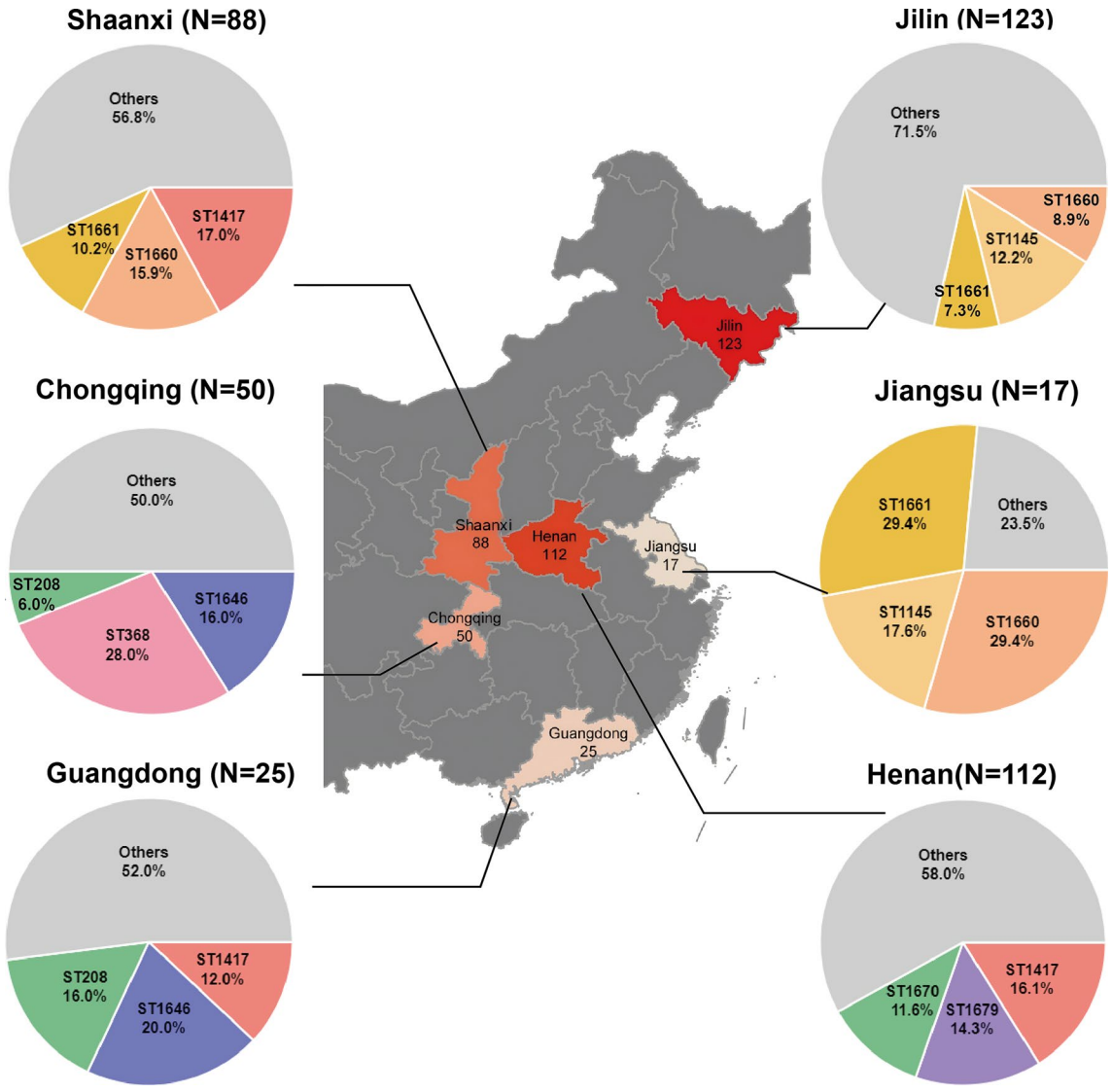

Fig. 2. Regional distribution of predominant *A. baumannii* sequence types across six Chinese provinces. Pie charts show the percentage distribution of major STs in each province. Numbers in parentheses indicate total isolates per province. "Others" includes remaining STs with lower frequencies. Maps show partial regions of China covering the six study provinces.

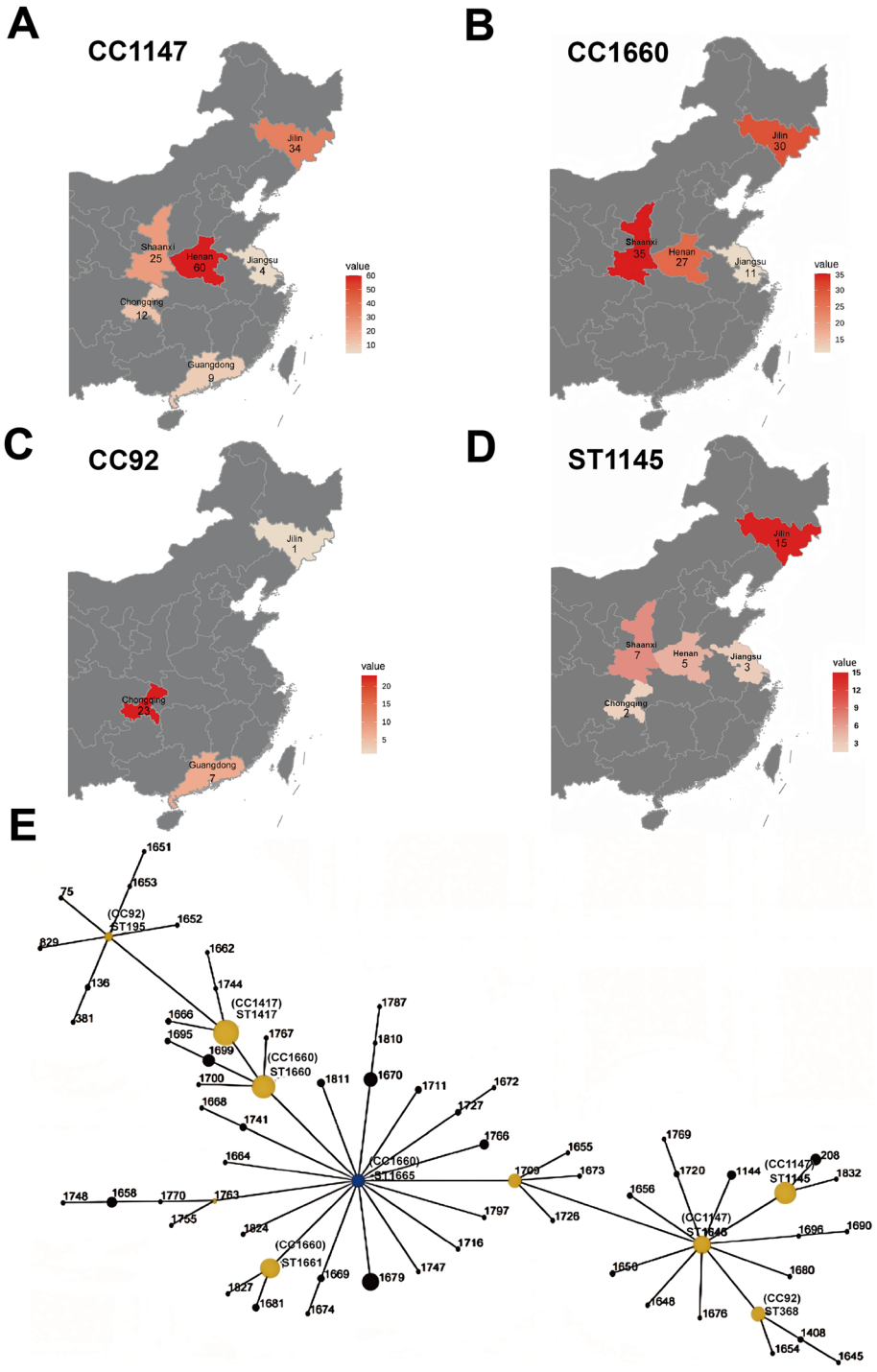

Fig. 3. eBURST analysis of 415 *Acinetobacter baumannii* isolates. (**A–D**) Geographical distribution maps of major clonal complexes and sequence type across six Chinese provinces. Maps show isolate density by province. (**A**) CC1417 distribution. (**B**) CC1660 distribution. (**C**) CC92 distribution. (**D**) ST1145 distribution. Maps show partial regions of China covering the six study provinces. (**E**) Relationships among the STs were found in this study. Connections between STs indicate single-locus variants (SLVs) differing by one allele at a single locus. The blue dot denotes the founder, and the yellow dots represent the sub-founder. The size of the dot indicates the number of isolates. Central node ST1646 corresponds to CC1417, central node ST1665 corresponds to CC1660, and central node ST195 corresponds to CC92. This network demonstrates the close evolutionary relationships within each clonal complex (CC), with each CC displaying a star-like radiation pattern from central founder STs to peripheral derivative STs.

Items	Regions						Total
	Chongqing	Guangdong	Jiangsu	Henan	Shaanxi	Jilin	
Total	50	25	17	112	88	123	415
SXT	44 (88%)	23 (92%)	10 (59%)	94 (84%)	64 (73%)	108 (88%)	343 (82.65%)
CIP	47 (94%)	22 (88%)	12 (71%)	105 (94%)	71 (81%)	123 (100%)	380 (91.57%)
GEN	47 (94%)	22 (88%)	11 (65%)	101 (90%)	72 (82%)	116 (94%)	369 (88.92%)
CAZ	46 (92%)	22 (88%)	11 (65%)	103 (92%)	73 (83%)	123 (100%)	378 (91.08%)
CSL	36 (72%)	16 (64%)	8 (47%)	71 (63%)	49 (56%)	108 (88%)	288 (69.40%)
CTX	49 (98%)	24 (96%)	12 (71%)	108 (96%)	75 (85%)	116 (94%)	380 (91.57%)
IPM	46 (92%)	23 (92%)	10 (59%)	103 (92%)	74 (84%)	116 (94%)	376 (90.60%)
PIP	48 (96%)	24 (96%)	13 (76%)	108 (96%)	76 (86%)	108 (88%)	377 (90.84%)
TGC	12 (24%)	2 (8%)	3 (18%)	15 (13%)	19 (22%)	93 (76%)	155 (37.35%)
MDR	47 (94.0%)	24 (96.0%)	15 (88.2%)	102 (91.1%)	80 (90.9%)	116 (94.3%)	384 (92.5%)
XDR	2 (4.0%)	3 (12.0%)	2 (11.8%)	8 (7.1%)	10 (11.4%)	13 (10.6%)	38 (9.2%)

Table 2. Resistance of regional isolates against selected antibiotics. SXT, sulfamethoxazole; CIP, Ciprofloxacin; GEN, Gentamicin; CAZ, Ceftazidime; CSL, Cefoperazone/sulbactam; CTX, Cefotaxime; IPM, Imipenem; PIP, Piperacillin; TGC, Tigecycline; MDR: Multidrug-resistant; XDR: Extensively drug-resistant. MDR (Multidrug-resistant): Non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories. In this study, defined as resistant to 3 or more antibiotic classes with inhibition zone diameter ≤ 14 mm. XDR (Extensively drug-resistant): Non-susceptible to ≥ 1 agent in all but ≤ 2 antimicrobial categories. In this study, defined as resistant to all tested antibiotics except 1–2 categories. Resistance breakpoint: Inhibition zone diameter ≤ 14 mm was considered resistant.

Antibiotics	Top six STs					
	ST1417	ST1660	ST1145	ST1661	ST1646	ST1679
SXT	36 (80%)	26 (72%)	23 (72%)	18 (69%)	17 (77%)	19 (95%)
CIP	40 (89%)	29 (81%)	26 (81%)	21 (81%)	17 (77%)	20 (100%)
GEN	40 (89%)	29 (81%)	26 (81%)	19 (73%)	20 (91%)	20 (100%)
CAZ	39 (87%)	28 (78%)	25 (78%)	21 (81%)	20 (91%)	18 (90%)
CSL	29 (64%)	19 (53%)	17 (53%)	17 (65%)	13 (59%)	14 (70%)
CTX	40 (89%)	31 (86%)	28 (88%)	21 (81%)	21 (95%)	19 (95%)
IPM	40 (89%)	30 (83%)	27 (84%)	21 (81%)	18 (82%)	19 (95%)
PIP	41 (91%)	30 (83%)	27 (84%)	22 (85%)	21 (95%)	20 (100%)
TGC	7 (16%)	16 (44%)	14 (44%)	9 (35%)	2 (9%)	7 (35%)

Table 3. Data of top six STs (sequence types) isolates resistance against selected antibiotics. ST: Sequence typing of multi locus sequence typing; SXT, Sulfamethoxazole; CIP, Ciprofloxacin; GEN, Gentamicin; CAZ, Ceftazidime; CSL, Cefoperazone/sulbactam; CTX, Cefotaxime; IPM, Imipenem; PIP, Piperacillin; TGC, Tigecycline

contrast to the others (Table 3). These six clones demonstrated a high degree of resistance towards a majority of antibiotics, yet exhibited a comparatively lower resistance towards TGC (Table 3).

Detection of resistance genes

The mechanisms and genes of drug resistance of carbapenems mainly include β-lactamase, multidrug efflux pump, and permeability defects. Through a comprehensive literature review, we identified 21 resistance genes. Interestingly, these were screened in all isolates by PCR. These genes were functionally categorized into three corresponding mechanisms: β-lactamase-encoding genes (including class A, B, C, and D β-lactamases), efflux pump-associated genes, and genes related to permeability defects.

For the top six STs, all of these isolates carried $bla_{\text{OXA-58}}$, bla_{IMP}, bla_{AmpC}, and *carO* (Table 4). However, the carrying rates of β-lactamase $bla_{\text{GES-14}}$, $bla_{\text{CTX-M-2}}$, bla_{KPC}, and $bla_{\text{SIM-1}}$ varied significantly. From the perspective of regional distribution, all 415 isolates carried four genes: bla_{AmpC}, $bla_{\text{OXA-58}}$, bla_{IMP}, and *carO* (Table 5). Meanwhile, over 85% of the strains from each province carried the following genes: bla_{VIM}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-51}}$, *omp33-36, adeA, adeB, adeC, adeI* and *adeJ* (Table 5). Only *ompW* and $bla_{\text{SIM-1}}$ had relatively low carrying rates (Table 5). The carrying rates of efflux pump class, *carO* and *omp33-36* of permeability defects, bla_{AmpC}, bla_{IMP}, bla_{NDM}, $bla_{\text{OXA-58}}$, $bla_{\text{OXA-23}}$, and $bla_{\text{OXA-51}}$ of β-lactases were generally high in six provinces (Table 5). There were substantial regional differences in the individual carrying rate of resistance genes. The proportion of the strains carrying *ompW* in Shaanxi was 76%, which was two to three times that of the other five regions (Table

Resistance gene			Top six STs					
			ST1417	ST1660	ST1145	ST1661	ST1646	ST1679
ß-Lactamases	Class A	bla_{GES-14}	37 (82%)	29 (81%)	31 (97%)	22 (85%)	22 (100%)	16 (80%)
		bla_{KPC}	36 (80%)	30 (83%)	29 (91%)	22 (85%)	22 (100%)	8 (40%)
		bla_{PER}	42 (93%)	31 (86%)	29 (91%)	24 (92%)	22 (100%)	20 (100%)
		$bla_{CTX-M-2}$	37 (82%)	28 (78%)	17 (53%)	16 (62%)	22 (100%)	19 (95%)
	Class B	bla_{IMP}	45 (100%)	36 (100%)	32 (100%)	26 (100%)	22 (100%)	20 (100%)
		bla_{VIM}	43 (96%)	33 (92%)	32 (100%)	22 (85%)	22 (100%)	19 (95%)
		bla_{SIM-1}	18 (40%)	22 (61%)	21 (66%)	19 (73%)	19 (85%)	8 (40%)
		bla_{NDM}	45 (100%)	34 (94%)	32 (100%)	26 (100%)	19 (85%)	19 (95%)
	Class C	bla_{AmpC}	45 (100%)	36 (100%)	32 (100%)	26 (100%)	22 (100%)	20 (100%)
	Class D	bla_{OXA-23}	45 (100%)	35 (97%)	32 (100%)	25 (96%)	22 (100%)	20 (100%)
		bla_{OXA-24}	33 (73%)	30 (83%)	28 (88%)	24 (92%)	20 (92%)	20 (100%)
		bla_{OXA-51}	45 (100%)	36 (100%)	32 (100%)	26 (100%)	22 (100%)	13 (65%)
		bla_{OXA-58}	45 (100%)	36 (100%)	32 (100%)	26 (100%)	22 (100%)	20 (100%)
Efflux pumps		*adeI*	45 (100%)	36 (100%)	32 (100%)	26 (100%)	19 (85%)	19 (95%)
		adeB	44 (98%)	36 (100%)	32 (100%)	25 (96%)	20 (92%)	20 (100%)
		adeA	44 (98%)	36 (100%)	32 (100%)	26 (100%)	20 (92%)	20 (100%)
		adeJ	43 (96%)	36 (100%)	32 (100%)	26 (100%)	20 (92%)	20 (100%)
		adeC	44 (98%)	36 (100%)	32 (100%)	26 (100%)	20 (92%)	19 (95%)
Permeability defects		*omp33-36*	44 (98%)	36 (100%)	32 (100%)	25 (96%)	20 (92%)	19 (95%)
		ompW	20 (44%)	15 (42%)	12 (38%)	15 (58%)	7 (31%)	4 (20%)
		carO	45 (100%)	36 (100%)	32 (100%)	26 (100%)	22 (100%)	20 (100%)

Table 4. Resistance gene carrying rate of the top six STs isolates. ST: Sequence typing of multi locus sequence typing.

Resistance gene			Regions					
			Chongqing	Guangdong	Henan	Jiangsu	Jilin	Shaanxi
ß-Lactamases	Class A	bla_{PER}	47 (94%)	18 (72%)	108 (96%)	14 (82%)	108 (88%)	82 (93%)
		bla_{GES-14}	46 (92%)	21 (84%)	87 (78%)	16 (94%)	79 (64%)	70 (80%)
		$bla_{CTX-M-2}$	49 (98%)	24 (96%)	106 (95%)	11 (65%)	73 (59%)	61 (69%)
		bla_{KPC}	44 (88%)	23 (92%)	67 (60%)	16 (94%)	65 (53%)	88 (100%)
	Class B	bla_{NDM}	47 (94%)	21 (84%)	108 (96%)	17 (100%)	116 (94%)	88 (100%)
		bla_{SIM-1}	36 (72%)	20 (80%)	45 (40%)	10 (59%)	101 (82%)	50 (57%)
		bla_{VIM}	49 (98%)	23 (92%)	112 (100%)	16 (94%)	123 (100%)	79 (90%)
		bla_{IMP}	50 (100%)	25 (100%)	112 (100%)	17 (100%)	123 (100%)	88 (100%)
	Class C	bla_{AmpC}	50 (100%)	25 (100%)	112 (100%)	17 (100%)	123 (100%)	88 (100%)
	Class D	bla_{OXA-23}	48 (96%)	22 (88%)	112 (100%)	17 (100%)	123 (100%)	84 (95%)
		bla_{OXA-24}	45 (90%)	20 (80%)	73 (65%)	14 (82%)	116 (94%)	76 (86%)
		bla_{OXA-51}	50 (100%)	25 (100%)	106 (95%)	17 (100%)	123 (100%)	88 (100%)
		bla_{OXA-58}	50 (100%)	25 (100%)	112 (100%)	17 (100%)	123 (100%)	88 (100%)
Efflux pumps		*adeA*	49 (98%)	22 (88%)	110 (98%)	16 (94%)	123 (100%)	88 (100%)
		adeB	49 (98%)	22 (88%)	111 (99%)	17 (100%)	123 (100%)	88 (100%)
		adeC	50 (100%)	22 (88%)	112 (100%)	17 (100%)	123 (100%)	88 (100%)
		adeI	50 (100%)	21 (84%)	109 (97%)	17 (100%)	123 (100%)	88 (100%)
		adeJ	50 (100%)	22 (88%)	109 (97%)	17 (100%)	123 (100%)	88 (100%)
Permeability defects		*omp33-36*	50 (100%)	22 (88%)	106 (95%)	17 (100%)	123 (100%)	88 (100%)
		ompW	18 (36%)	12 (48%)	27 (24%)	8 (47%)	30 (24%)	67 (76%)
		carO	50 (100%)	25 (100%)	112 (100%)	17 (100%)	123 (100%)	88 (100%)

Table 5. Regional resistance gene carrying rates.

5). Meanwhile, the bla_{SIM-1} carriage rate in Henan was considerably lower than in other regions. The $bla_{CTX-M-2}$ carrying rate is low in Jilin, Jiangsu and Shaanxi while the rate in the other provinces was more than 90% (Table 5). These findings suggest complex regional differences in selection pressures and resistance evolution patterns.

Discussion

Acinetobacter baumannii (*A. baumannii*), an opportunistic pathogen, mainly causes hospital-acquired infection, and it has been considered a critical-priority pathogen by the World Health Organization. An open pan-genome makes obtaining new genes and functions easier for *A. baumannii*. To characterize the regional distribution patterns and genetic evolution of *A. baumannii* and provide more references for the application of therapeutic antibiotics, the current study analyzed the molecular epidemiology of 415 *A. baumannii* isolates obtained in China from 2016 to 2020, including drug resistance, drug-resistant genes, and STs. These isolates were recovered from 6 provinces in China, including Jilin, Guangdong, Henan, Shaanxi, Jiangsu, and Chongqing. These provinces encompass the eastern, western, southern, and northern territories, encapsulating the geographical features prevalent throughout the nation[41]. This extensive coverage enables an in-depth exploration of regional variations and the influence of geographical environments on *A. baumannii* prevalence. Additionally, the six provinces demonstrate notable differences in population structure, economic development, healthcare standards, and infection control policies related to drug-resistant bacteria treatment[42,43]. These human-associated differences are well-documented in previous studies, which have shown significant regional disparities in health service development across Chinese provinces, with eastern regions (Guangdong and Jiangsu in our study) consistently demonstrating higher levels in healthcare personnel, facilities, and service provision compared to central (Henan and Shaanxi) and western regions (Chongqing)[42,43]. These variations are crucial for understanding the factors that contribute to the prevalence of *A. baumannii*.

Recently, extensively drug-resistant (XDR) and pan-drug-resistant (PDR) *A. baumannii* isolates have rapidly increased[44]. Consistent with this trend, antimicrobial susceptibility testing showed that 92.5% and 9.2% of burn wound *A. baumannii* isolates were MDR and XDR, respectively, posing significant therapeutic challenges. These findings align with previous studies in China, where carbapenem resistance rates reached 91–94% in Chongqing burn ICU (2018–2020)[56] and 92.3% in burn-related bloodstream infections[2].

Regional analysis revealed varying resistance patterns across provinces. Guangdong showed the highest MDR (96.0%) and XDR (12.0%) rates, while Jiangsu and Chongqing reported the lowest MDR (88.2%) and XDR (4%) rates, respectively (Table 2). These differences may reflect variations in healthcare infrastructure, antibiotic usage, and infection control practices[42,43], though specific determinants require further investigation. Among the tested antimicrobials, most *A. baumannii* isolates showed high resistance to tested antibiotics, except for tigecycline (TGC) and cefoperazone/sulbactam (CSL) (Table 2). This finding is particularly significant given the limited therapeutic options available for MDR *A. baumannii* infections. The resistance mechanisms and genes of *A. baumannii* include β-lactamases, modifications of target sites, permeability defects, efflux pumps, and aminoglycoside-modifying enzymes. β-lactamase genes encode β-lactamase enzymes that hydrolyze the β-lactam ring in antibiotics, resulting in antibiotic inactivation and conferring β-lactam resistance to *A. baumannii*. Different countries have reported that class D contributes the most to drug resistance β-lactamase in recent years, and the prevalence of class D in China can reach 90%, especially in coastal areas[45]. Through a comprehensive literature review, we identified 21 resistance genes spanning β-lactamases, membrane permeability factors, and efflux pump systems[46,47]. These genes were screened in all isolates by PCR. The systematic categorization of these resistance determinants allowed us to examine the complex interplay between different resistance mechanisms.

A previous study has shown a significant correlation between the overexpression of the AdeABC and AdeIJK efflux pumps and resistance to tigecycline and minocycline[48]. Despite, the high prevalence of efflux pump genes in the 415 strains, they remained susceptible to Tigecycline (TGC), suggesting that the efflux pumps in these strains may not be involved in resistance to TGC, or their expression levels may not be sufficient to confer significant resistance. Alternatively, these strains may possess other susceptibility mechanisms, such as unknown resistance inhibitory factors, or TGC may enter the cells in a manner that is not affected by these efflux pumps. Further studies are needed to confirm these possibilities and fully understand the complex interplay between efflux pump gene carriage and antibiotic susceptibility in these strains.

Here, MLST analysis was performed using the updated Oxford scheme primers as described by Hamidian et al. (2017) to avoid previous misidentification issues associated with the original primers, particularly for the gpi gene[49]. This revision is specifically important for accurate identification of sequence types, notably the correct identification of ST208 which was previously misidentified as ST92 due to primer design issues. While the updated Oxford MLST scheme has certain limitations, particularly regarding homologous recombination effects, it remains valuable for differentiating isolate relatedness within clonal groups. Although the Pasteur scheme is increasingly preferred for clonal group classification, the Oxford scheme's established presence in historical datasets makes it useful for comparative analyses across studies.

In our analysis, we employed the updated Oxford scheme to maintain consistency with existing literature and facilitate broader comparability of our findings. Due to the different environments and antibiotic selection, time and regional differences may exist in the prevalence of STs. Previous epidemiological data indicated that ST208 (initially misreported as ST92 due to limitations in the original Oxford MLST scheme) predominated in China before 2010, with ST208 and ST195 continuing to be the major epidemic types of MDR *A. baumannii* in recent years[1,50]. Regional variations in strain dominance were observed across different geographical locations, with ST208 and ST368 being prevalent in Chongqing, while ST208 (documented as ST92 in earlier studies) was predominantly identified in Henan[51,52]. Notably, a 12-year longitudinal epidemiological study of *A. baumannii* in a tertiary hospital documented a temporal shift in strain dominance: the originally prevalent ST208 and ST369 clones were replaced by ST451 in 2019[53].Consistently, our current investigation identified ST1417, ST1660, and ST1145 as emerging dominant sequence types, indicating a notable shift in regional epidemiological patterns.

These findings collectively suggest an ongoing clonal replacement phenomenon among predominant *A. baumannii* strains in China. This dynamic evolutionary pattern underscores the remarkable genomic plasticity of *A. baumannii* populations across spatial and temporal dimensions.

While our collected main ST types (ST1417, ST1660, and ST1145) are different from a recent epidemiological study (2011–2021) focusing on *A. baumannii* blood isolates (ST208 and ST191 as dominant)[1], the difference exemplifies the complex, multifactorial nature of *A. baumannii* molecular epidemiology. Previous research has demonstrated that regional variations in *A. baumannii* epidemiology result from the combined effects of geographical isolation, diverse transmission pathways, differential antibiotic selection pressures, and continuous bacterial evolution[25]. Beyond these established factors, we propose that clinical source materials may represent an additional determinant of strain distribution patterns. The referenced study primarily sampled from Zhejiang (40.23%) and Anhui (19.99%) provinces[1], excluding Chongqing and Jilin, which were key regions in our investigation. Moreover, their samples were blood isolates[1] rather than wound samples. However, the differences also suggest that *A. baumannii* evolves rapidly, highlighting the need for epidemiological surveillance. These distinctions in geographical coverage, sampling density, and strain sources account for the differences in our findings.

Previous studies on *A. baumannii* isolates from burn patients in China have documented distinct epidemiological patterns. In a burn ICU study in Chongqing (2013), ST368 emerged as the dominant sequence type, along with ST369, ST195, and ST191[54]. Similarly, research on burn-related bloodstream infections at the Chinese Burn Institute identified ST368 as the most prevalent sequence type (38%)[2]. These studies consistently reported high carbapenem resistance rates (91–94%) in burn isolates[2,54]. In contrast, our current investigation identified ST1417, ST1660, and ST1145 as the predominant sequence types in burn wound isolates, suggesting temporal and geographical variations in dominant clones. This further emphasizes the critical importance of continuous epidemiological surveillance in tracking the dynamic shifts in dominant strains in burn infections across regions and time periods. The emergence of different predominant strains in burn infection in our study underscores the evolving nature of A. *baumannii* epidemiology and reinforces the necessity for sustained, long-term monitoring to effectively track these changes.

Regarding resistance gene profiles, the Chongqing burn ICU study detected *blaOXA-51* (100%), *blaOXA-23* (83.9%), *blaVIM* (70.9%), *blaAmpC* (80.6%), and *blaPER* (40.3%) in patient isolates[54]. Our study showed a similarly high prevalence of *blaOXA-51* (100%) and *blaOXA-23* (96%), but notably higher rates of *blaVIM* (98%), *blaAmpC* (100%), and *blaPER* (94%) (Table 5). Additionally, our isolates demonstrated widespread presence of *blaNDM* (94%) and *blaIMP* (100%), which were not detected in the earlier Chongqing study (Table 5). These differences likely reflect the temporal evolution of resistance mechanisms and the increasing prevalence of metallo-β-lactamases in Chinese hospitals over the past years.

Our study provides the first comprehensive evidence of CC1417 (34.7%) and CC1660 (24.8%) emergence as major clones in burn wound isolates from several provinces of China. CC92 has been a widely prevalent clonal complex globally[55]. A study reported that among 92 isolates collected in the United States from 2013 to 2017, the majority (n = 59, 64%) belonged to clonal complex 92 (CC92)[56]. The dominance of CC92 has been documented in epidemiological studies in Malaysia and Mexico as well[57,58]. However, in 2023, a study collected 57 carbapenem-resistant *Acinetobacter baumannii* strains, with more than half (56.1%, 32/57) belonging to CC455 and over one-third (38.6%, 22/57) belonging to CC92 [59]. Compared to previous studies, our study indicates that CC1417 and CC1660, rather than CC92, have become the predominant strains in burn wound isolates from several provinces of China. Moreover, it is noteworthy that CC92 remains dominant in Chongqing, which is also the main distribution area of all CC92 strains. This may reflect clonal stability or distinct local selection pressures in Chongqing. The geographical analysis of ST1145 shows uneven distribution across provinces (Fig. 3D). ST1145 was more frequently detected in Jilin (12.2%), detected at lower levels in Jiangsu, and not detected in Guangdong (Fig. 3D).

The epidemiological investigation of *A. baumannii* holds substantial importance for the advancement of precision medicine. While precision medicine is generally considered to be related to cancer detection and treatment, employing advanced genomic, epigenomic, and other omics technologies alongside targeted molecular drugs, it often encounters limitations due to socioeconomic disparities across regions[60]. Investigating the epidemiology of *A. baumannii* can reveal risk factors for infection in diverse regions, populations, and environments, thereby informing targeted preventive measures to mitigate infection risks in specific populations. This study predicts that the predominant infection type of *A. baumannii* burn wound isolates in Chongqing or Southwest China is CC92, indicating the need for increased attention and research on CC92 in this region. Drug resistance in *A. baumannii* may exhibit variation across different regions and populations. The carrying rates of $bla_{\text{SIM-1}}$ from Henan isolates and $bla_{\text{CTX-M-2}}$ from Jilin, Jiangsu and Shaanxi isolates were found to be low, indicating that their drug resistance may be less associated with β-lactamase pathway. Notably, the proportion of strains carrying *ompW* in Shaanxi Province reached 76%, which is two to three times higher than that of the other five regions. *OmpW* has been identified as a virulence factor in *A. baumannii*, and its absence compromises bacterial adhesion and invasion of host cells while reducing biofilm formation[61]. Of particular significance, previous studies have demonstrated that *ompW* gene expression is upregulated during the development of colistin resistance in *A. baumannii*[62]. Thus, research on *OmpW* and its associated drug therapies holds substantial implications for the treatment of *A. baumannii* infections in Shaanxi Province. These regional variations in resistance mechanisms likely reflect distinct local selection pressures and underscore the necessity for region-specific treatment strategies.

The present investigation has several notable limitations. First, this study focused exclusively on burn wound isolates, which may limit the generalizability of findings to other types of *A. baumannii* infections. The unique environment of burn units and the specific patient population may select for particular bacterial lineages that differ from those found in other clinical settings. Although data from the six provinces provides valuable insights

into regional patterns, the sample scope needs further expansion to establish a more comprehensive national profile. Additionally, the unbalanced distribution of isolates among provinces (ranging from 17 to 123 isolates per province) may affect the representativeness of regional comparisons. Moreover, a significant methodological limitation lies in our reliance on PCR-based detection of resistance genes which only identifies target resistance determinants rather than a full spectrum of genetic elements that whole-genome sequencing might reveal[40]. Furthermore, it is noteworthy that the quantity of strains gathered in each provinces is relatively insufficient, and thus may not be entirely representative, reminding us that epidemiological surveillance and genetic evolution analysis of *A. baumannii* is of great importance to provide more insights for the prevention of nosocomial infection and clinical treatment.

Conclusion

This molecular epidemiological investigation reveals significant shifts in the landscape of *A. baumannii* burn wound infections in selected provinces of China. Our findings demonstrate that CC1417 (34.7%) and CC1660 (24.8%) have emerged as predominant clonal complexes in burn wound isolates from several provinces of China during the study, showing different distribution patterns compared to previously reported dominant CC92, with distinct regional distribution patterns. The concomitant identification of ST1417, ST1660, and ST1145 as prevalent sequence types in burn wound infections reinforces this epidemiological transition, with ST1145 showing higher detection in northern provinces, reflecting uneven regional distribution. Our analysis demonstrates high prevalence of antimicrobial resistance, with 92.5% of isolates classified as MDR and 9.2% as XDR. Significant regional variations in resistance patterns were observed across provinces, with MDR rates ranging from 88.2% to 96.0% and XDR rates from 4 to 12.0%. However, the relatively preserved susceptibility to tigecycline (TGC) and cefoperazone/sulbactam (CSL) offers potential therapeutic options. The high prevalence of multiple resistance genes, including *β-lactamases*, efflux pumps, and permeability-related genes, combined with significant regional variations in resistance mechanisms, highlights the dynamic evolution of this pathogen. Of particular concern is the regional specificity of certain resistance patterns, such as the elevated *ompW* carriage in Shaanxi (76%) and variable distribution of *β-lactamase* genes across provinces. These findings specifically apply to burn wound *A. baumannii* isolates in selected provinces and underscore the importance of continued surveillance and molecular typing in tracking the evolution and spread of resistant strains.

Data availability

Due to the nature of MLST data, which often contains numerous repeated sequences and minor variations that don't significantly impact the final typing results, these sequence data are not typically stored in public databases. However, to ensure maximum transparency, we have uploaded our 415 isolates' ST typing data to PubMLST at: https://pubmlst.org/bigsdb?db=pubmlst_abaumannii_isolates&l=1&page=query and data will be provided upon request from corresponding author (Email: zichenyang@tmmu.edu.cn).

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Author contributions

SP was responsible for manuscript writing, data analysis, and figure preparation. ZL and ZY jointly supervised the project, contributed to experimental design, and participated in data analysis and figure preparation. KL, YH, YG, and JW performed bacterial isolation and cultivation procedures. ZL and ZY conceptualized the study and critically revised the manuscript. All authors have read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval and informed consent

This is an original research article on bacteria sequencing data. For this type of study, the requirement for ethics approval was approved by the Medical Ethics Committee of the Second Affiliated Hospital (Xinqiao Hospital) of Army Medical University, PLA (Approval No.2016-170-01). For this particular type of research, the requirement for informed consent was waived.

Additional information

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