

# Molecular Characterization and Antibiotic Resistance of *Acinetobacter baumannii* in Cerebrospinal Fluid and Blood

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## Research

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# Abstract

## Background

The increasing rates of carbapenem-resistant *Acinetobacter baumannii* (CRAB) caused nosocomial infections generate significant comorbidity and sometime cause death among patients. Current treatment options are limited. These infections pose great difficulties for infection control and clinical treatment. This study identifies the antimicrobial resistance, carbapenemases and genetic relatedness of *A. baumannii* isolates from cerebrospinal fluid (CSF) and blood in a hospital in Shandong, China.

## Methods

A total of 50 nonrepetitive CSF *A. baumannii* isolates and 44 blood isolates were collected. The resistance phenotypes were determined according to the Clinical and Laboratory Standards Institute guidelines. We performed Polymerase Chain Reaction (PCR) experiments to detect the carbapenem resistance mechanism. Finally, we conducted Multilocus Sequence Typing (MLST) to depict the genetic relatedness of these isolates.

## Results

We observed that eighty-eight of the 94 isolates collected were resistant to imipenem or meropenem. Among them, the bla OXA-23 gene was the most prevalent carbapenemase gene with a 91.5% (86/94) detection rate, followed by the bla OXA-24 gene that showed a 2.1% (2/94) detection rate isolates. Among all CRAB observations in this study, isolates with the bla OXA-23 gene were resistant to both imipenem and meropenem. However, isolates positive for the bla OXA-24 gene but negative for the bla OXA-23 gene showed an imipenem-sensitive but meropenem-resistant phenotype. The outcome of multilocus sequence typing analysis showed 21 different STs were distinguished, of which ST195 (25.5%), ST540 (12.8%) and ST208 (11.7%) were most frequently observed. Eighty of the 94 isolates (85.1%) were clustered into CC92, and all CC92 isolates showed a carbapenem resistance phenotype (except AB13). Five novel STs were detected, and most of them were CRAB, some of which belonged to CC92.

## Conclusion

A high level of carbapenem resistance was detected in this study. The CC92 and bla OXA-23 gene were predominant. Five novel STs were detected, and these new STs require further investigation to understand the nature of and to prevent outbreaks caused by *A. baumannii*. Our study provides additional observations and epidemiological data of CSF and blood *A. baumannii* strains, which may improve future infection control measures and aid in potential clinical treatment in hospitals and other clinical settings.

## Background

*Acinetobacter baumannii* is a nonfermentative, gram-negative opportunistic pathogen that often causes disease among immunocompromised patients [1]. *A. baumannii* is often found in hospitals causing a

variety of nosocomial infectious diseases, including bloodstream infections, urinary tract infections, meningitis and wound infections [2]. In recent years, *A. baumannii* has become an important bacterium to identify when treating and controlling infectious diseases because of its remarkable ability to evolve extensive drug resistance to many antibiotics [3].

Some observations are referred to as Critical Values because these laboratory values may indicate an urgent and a life-threatening situation for patients where treatment protocols indicate immediate therapy must be initiated. This includes microbiology results identifying the bacteria in cerebrospinal fluid (CSF) and blood [4, 5]. Therefore, the presence of *A. baumannii* strains in CSF and blood is a Critical Value to identify for practitioners as it can poses great difficulties for clinical treatment options.

Carbapenems are considered to be the most effective antibiotics against many multidrug-resistant bacteria [6]. However, the increase in carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates has recently become a global concern. CHINET surveillance data showed that from 2005 to 2018, the resistance of *A. baumannii* to imipenem and meropenem increased approximately twofold [7, 8]. The production of carbapenemase is one of the most common and important mechanisms for *A. baumannii* resistance to carbapenems. Among all carbapenemases, OXA-type (mainly OXA-23, OXA-24, OXA-48, OXA-51 and OXA-58) was the most prevalent [9-11]. In addition, because the coding genes are located in transferable genetic elements and can spread among *A. baumannii* and even into other bacteria [12, 13], New Delhi metallo- $\beta$ -lactamase (NDM) and *Klebsiella pneumoniae* carbapenemase (KPC) producers have also shown significant importance for worldwide prevalence [14, 15].

The multilocus sequence typing (MLST) method has been widely used to depict genetic relatedness and for molecular epidemiological studies of *A. baumannii* [16]. Previous molecular epidemiological studies have shown that CC92 is highly prevalent throughout China, and it is the clonal complex (CC) with the widest global distribution [17, 18].

In this work, we observe, investigate and characterized 94 *A. baumannii* isolates obtained from CSF and blood we acquired from patients in university hospital in Shandong, China between 2014 and 2019.

## Results

### Distribution of bacterial isolates

*A. baumannii* isolates were collected from two different locations, CSF (n=50) and blood (n=44), from the First Hospital Affiliated with Shandong First Medical University. For the CSF isolates, one was collected in 2014, four in 2015, seventeen in 2016, fifteen in 2017, five in 2018 and eight in 2019. Forty-one of these isolates were obtained from neurosurgery, six from the ICU and three from other wards. Regarding the *A. baumannii* isolates samples obtained from blood; twelve were collected in 2016, thirteen in 2017, eleven in 2018 and eight in 2019. Twenty-eight blood isolates were obtained from the ICU, six from neurosurgery, four from hematopathology and six from other wards.

# Antibiotic susceptibility

The antimicrobial susceptibility profiles of 94 *A. baumannii* strains were shown in Fig.1 and Table S1 in Supplemental Material. More than 90% of the *A. baumannii* isolates were resistant to ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime and ciprofloxacin. A total of 77.7% of the isolates were resistant to tobramycin, 71.3% were resistant to levofloxacin, 59.6% to trimethoprim/sulfamethoxazole and 52.1% to cefepime. The resistance rate for minocycline and cefoperazone/sulbactam, which are commonly used for CRAB infection, were 19.1% and 45.7% respectively. In contrast, only seven isolates (7.4%) were resistant to tigecycline and all isolates were sensitive to colistin. Among all 94 *A. baumannii* isolates, 86 (93.6%) isolates were resistant to both imipenem and meropenem. Two isolates showed a meropenem-resistant but imipenem-sensitive phenotype.

## Distribution of carbapenem resistance genes

All isolates were detected for the presence of carbapenem resistance genes (Table 1). In the 94 *A. baumannii* isolates, all of them harbored the intrinsic bla<sub>OXA-51</sub> gene. In contrast, none of them had bla<sub>OXA-48</sub>, bla<sub>OXA-58</sub>, NDM-1 or KPC genes. The bla<sub>OXA-23</sub> gene was the most prevalent carbapenemase gene with a 91.5% (86/94) detection rate, followed by the bla<sub>OXA-24</sub> gene in only 2 (2.1%) isolates.

In this study, all of the isolates with bla<sub>OXA-23</sub> or bla<sub>OXA-24</sub> genes were carbapenem resistant. Among them, isolates with the bla<sub>OXA-23</sub> gene were resistant to both imipenem and meropenem. However, isolates positive for the bla<sub>OXA-24</sub> gene but negative for the bla<sub>OXA-23</sub> gene showed an imipenem-sensitive but meropenem-resistant phenotype.

Table 1  
Positive rates of carbapenem resistance genes

No. of strains	Rate of gene (%)						
	OXA-23	OXA-24	OXA-48	OXA-51	OXA-58	NDM1	KPC
94	91.5	2.1	0	100	0	0	0

## MLST profile

The MLST analysis revealed a total of 21 different STs, including 16 existing STs and 5 novel STs we identified in this study (the new STs were submitted for ST assignment which were ST1967, ST1968, ST1969, ST1970 and ST1971). The profiles of the newly identified ST types are listed in Table 2. Among

them, 89.4% (84/94) were represented by 11 main STs (having  $\geq 2$  isolates), and the prevalent STs were ST195, ST540 and ST208, accounting for 25.5% (24/94), 12.8% (12/94), and 11.7% (11/94), respectively (Fig.2 and Table S2 in the supplemental material).

Twelve STs representing 85.1% (80/94) of the isolates were clustered into CC92, with up to 18 different allelic profiles and 56 different isolates being represented. In addition, 9 individual STs accounted for 14 isolates (The detailed MLST profiles can be seen in Table S2 in Supplemental Material).

**Table 2 Allelic profiles of the new STs found in this study**

STs	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>
1967	1	34	3	2	2	178	3
1968	1	3	3	2	2	113	3
1969	1	17	135	12	23	98	6
1970	1	3	3	2	2	160	4
1971	36	34	59	28	4	279	3

## MLST, antibiotic susceptibility and carbapenem resistance genes

All of the isolates grouped into CC92 were carbapenem-resistant *A. baumannii* except for one isolate (AB13). These isolates were also not sensitive to ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefepime, ciprofloxacin and levofloxacin but had variable susceptibilities to cefoperazone/sulbactam, tobramycin, minocycline, tigecycline and trimethoprim/sulfamethoxazole. In contrast, all of the CSAB isolates belonged to individual STs except for one isolate (AB13). These isolates were also sensitive to the other 12 antibiotics tested in this study.

Five novel STs were identified in this study. All 4 ST1967 were isolated from CSF and were non-sensitive to all  $\beta$ -lactam antibiotics and quinolones. For ST 1968 isolates, 5 were non-sensitive to 13 antibiotics (except colistin) tested in this study, but the other one isolate (AB73) was sensitive to tobramycin, trimethoprim/sulfamethoxazole and colistin. ST1970 isolate was non-sensitive to 12 antibiotics (except tigecycline and colistin) tested in this study, whereas the ST1971 isolate was sensitive to all antibiotics. ST1969 contained two isolates (AB29 and AB70). Both of these isolates possessed the bla<sub>OXA-24</sub> gene and were resistant to meropenem but sensitive to imipenem.

## Discussion

CSF and blood infection of *A. baumannii* (especially for CRAB) may be life-threatening and bring great obstacles for clinical treatment [4, 5]. This study offers insight into the molecular characterization and antibiotic resistance of *A. baumannii* from CSF and blood.

In China, from 2005 to 2018, the resistance rate of *A. baumannii* for imipenem and meropenem increased from 32.9% to 71.7% and from 41.3% to 78.1%, respectively [7, 8]. Compared to the CHINET surveillance data, the resistance rates of *A. baumannii* for imipenem and meropenem in our experiment were 91.5% and 93.6%, respectively, higher than the surveillance data. For CSF and blood infection, the recommended dose of antibiotics was usually higher and needed a longer course of treatment than the case of superficial infection. However the CHINET surveillance data contained antibiotic resistance data for bacteria isolated from various sites, including some superficial infections. This might be the cause of the higher drug resistance rate in our study. In addition, the CHINET surveillance data [7, 8, 19, 20] demonstrated that the resistance rate of *A. baumannii* to meropenem is slightly higher than that to imipenem. This is similar to our observations and results. In fact, our results also support the view that imipenem is more bactericidal [21] and has a higher T>MIC value [22] than meropenem against *A. baumannii*. In contrast, the resistance rate to tigecycline was only 7.4%, and all isolates showed colistin sensitive phenotype. The resistance rates of these two drugs were far below carbapenem and the other antibiotics tested in this study, suggesting that these two drugs might serve as therapeutic agents to control infections.

To investigate the mechanism of carbapenem resistance, carbapenemase-encoding genes were tested in this study. Our results showed that the bla<sub>OXA-23</sub> gene existed in most CRAB isolates but was absent for most CSAB isolates, which indicated that bla<sub>OXA-23</sub> was the major mechanism for carbapenem resistance of CRAB isolated from CSF and blood. The bla<sub>OXA-23</sub> gene was also the most important mechanism for carbapenem resistance in CRAB in China [18, 23] and some other countries [24-26]. On the other hand, the bla<sub>OXA-24</sub> gene was another mechanism for carbapenem resistance, as bla<sub>OXA-24</sub>-positive but bla<sub>OXA-23</sub>-negative isolates in this study showed meropenem-resistance and imipenem-sensitive characteristics. bla<sub>OXA-24</sub>-positive *A. baumannii* strains have been reported in many countries [9, 27, 28], especially in Spain, where bla<sub>OXA-24</sub> was the most prevalent gene [9]. Interestingly, even though most bla<sub>OXA-24</sub>-positive isolates have been reported to be resistant to both imipenem and meropenem, in our experiment, bla<sub>OXA-24</sub>-positive strains showed an imipenem-sensitive but meropenem-resistant phenotype. Some molecular biological mechanisms have been reported in many gram-negative bacteria to explain the imipenem-sensitive but meropenem-resistant phenomenon. For example, the transmission of the bla<sub>IMP-6</sub> and bla<sub>CTX-M-2</sub> plasmids [29] as well as the absence of OmpK35 and the frame shift mutation in OmpK36 [30] have been shown to be important mechanisms for imipenem-sensitive but meropenem-resistant *Klebsiella pneumoniae* (ISMRKP) strains. Substrate specificities of efflux pumps lead to different drug resistance characteristics for *Pseudomonas aeruginosa*. As a specific substrate, meropenem could be extruded by many efflux pumps, but imipenem was not affected by these efflux systems [31]. As a result,

some imipenem-sensitive but meropenem-resistant *Pseudomonas aeruginosa* strains were detected. However, studies examining this mechanism against *A. baumannii* are scarce. Both of these strains belonged to a novel ST (ST1969). This molecular mechanism for *A. baumannii* strains will require further investigation.

ST540, ST195 and ST208 were three major STs for *A. baumannii* isolated from CSF and blood. Among them, ST195 and ST208 are two dominant STs currently found in China [32-34]. Although ST540 was not the main ST observed in China, it was shown that ST540 was not only one of the three common STs but also the predicted founder of the CC for *A. baumannii* isolated from blood and CSF. On a global scale, CC92 was the largest and most geographically diverse CC, which was widespread in many countries [34], including China [23]. Although we did not find ST92 isolates (the predicted founder of CC92) in this study, it was shown that most *A. baumannii* isolated from CSF and blood belonged to CC92 (CC92 was the unique CC clonal group tested). In addition, 79 of 80 CC92 isolates were CRAB (except for one isolate, AB13), whereas only approximately 50% of the CC92 isolates were resistant to imipenem or meropenem, which suggested that CC92 isolates tend to acquire carbapenem resistance determinants. A previous study reported that CC92 is a widespread variant that has advantages in acquiring resistance determinants and surviving in the nosocomial environment, which renders it preferentially selected under antibiotic pressure [18]. This is a possible reason for the high detection rate of CC92 *A. baumannii* isolates in CSF and blood and the high correlation between CC92 and carbapenem resistance characteristics.

We also observed a total of five new STs. Among them, two novel STs were classified into CC92 and others were individual STs, which suggested that *A. baumannii* isolates were diverse and still in clonal expansion. As 13 of the 14 isolates in the five new STs were identified as CRAB, close attention should be paid toward these new STs to identify and further limit both transmission and outbreaks.

## Conclusion

In summary, with our study, we described the molecular characterization and antibiotic resistance of *A. baumannii* from CSF and blood in a hospital in Shandong, China. A high level of carbapenem resistance was detected. The CC92 and bla<sub>OXA-23</sub> gene were predominant in this hospital. Five novel STs were detected, and most of them were CRAB, some of which belonged to CC92. This study offers new epidemiological data of CSF and blood *A. baumannii* strains, which may help to improve infection control measures and clinical treatment in hospitals.

## Methods

### Bacterial isolates

A total of 94 nonrepetitive *A. baumannii* isolates were obtained from CSF or blood samples of patients from different departments (neurosurgery, ICU, hematopathology and other wards) at the First Hospital



Affiliated with Shandong First Medical University (Shandong, China). These samples were obtained from 2014 to 2019. All isolates were identified using MALDI-TOF MS (Bruker) and further verified by PCR products of 16S rDNA sequencing [35]. PCR products were sequenced by TsingkeBioTech Co., Ltd., followed by sequence alignment on the NCBI database.

## Antimicrobial susceptibility test

All *A. baumannii* strains were tested for susceptibility to 14 antibiotics, including ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, cefepime, imipenem, meropenem, tobramycin, ciprofloxacin, levofloxacin, minocycline, tigecycline, colistin and trimethoprim/sulfamethoxazole by using a Vitek 2 compact system (bioMérieux, Marcy, France) with AST-N-335 cards. The results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) criteria except for tigecycline were adapted from the United States Food and Drug Administration breakpoints.

## PCR Experiments

PCR assays were carried out using conventional PCR amplification. The target genes included the bla<sub>OXA-51</sub>, bla<sub>OXA-23</sub>, bla<sub>OXA-24</sub>, bla<sub>OXA-58</sub>, bla<sub>OXA-48</sub>, bla<sub>NDM-1</sub>, and bla<sub>KPC</sub> genes. Table 3 shows the sequences used for primer design and the annealing temperatures. The reaction conditions of the PCR programs consisted of an initial elongation at 94°C for 5 minutes; followed by 30 cycles of 94°C for 30 seconds, the respective annealing temperatures (Table 3) for 30 seconds, and 72°C for 1 minute; and a final extension step at 72°C for 10 minutes.

Table 3  
Primers used with their respective annealing temperatures.

Primer	Sequence	Amplicon length (bp)	Annealing temp (°C)	Ref
OXA-51-F	5'-ATGAACATTAAAGCACTC-3'	353 bp	46 °C	[36]
OXA-51-R	5'-CTATAAAATACCTAATTGTTC-3'			
OXA-23-F	5'-GATCGGATTGGAGAACCAGA-3'	501 bp	53 °C	[37]
OXA-23-R	5'-ATTTCTGACCGCATTTCCAT-3'			
OXA-24-F	5'-GGTTAGTTGGCCCCCTTAAA-3'	246 bp	53 °C	[37]
OXA-24-R	5'-AGTTGAGCGAAAAGGGGATT-3'			
OXA-58-F	5'-AAGTATTGGGGCTTGTGCTG-3'	599 bp	53 °C	[37]
OXA-58-R	5'-CCCCTCTGCGCTCTACATAC-3'			
KPC-F	5'-GCTCAGGCGCAACTGTAAGT-3'	823 bp	55 °C	[38]
KPC-R	5'-GTCCAGACGGAACGTGGTAT-3'			
NDM-1-F	5'-TCTCGACATGCCGGGTTTCGG-3'	475 bp	55 °C	[38]
NDM-1-R	5'-ACCGAGATTGCCGAGCGACTT-3			
OXA-48-F	5'-GCGTGGTTAAGGATGAACAC-3'	438 bp	52 °C	[39]
OXA-48-R	5'-CATCAAGTTCAACCCAACCG-3'			

## Multilocus Sequence Typing (MLST)

Multilocus sequence typing (MLST) analyses were performed using the Oxford scheme [16], publicly available from the [https://pubmlst.org/abaumannii/info/primers\\_Oxford.shtml](https://pubmlst.org/abaumannii/info/primers_Oxford.shtml). This MLST scheme is based on the sequencing of fragments of seven housekeeping genes: citrate synthase (*gltA*), DNA gyrase subunit B (*gyrB*), glucose dehydrogenase B (*gdhB*), homologous recombination factor (*recA*), 60-kDa chaperonin (*cpn60*), glucose-6-phosphate isomerase (*gpi*), and RNA polymerase sigma factor (*rpoD*).

Amplification reactions were carried out as described previously [18, 23], and sequencing was performed in both directions.

The sequences were aligned with the reference sequence from the MLST database (<https://pubmlst.org/abaumannii/>) using MEGA (version 4.0). BioEdit (version 7.0.1) was used to determine the allele assignments of the housekeeping genes before composing a profile of each strain. The newly identified STs were submitted to the MLST database curator for approval, and a number was assigned. A minimum-spanning tree using the allelic difference between isolates of the seven housekeeping genes was constructed using BioNumerics (Applied Math).

## Abbreviations

CRAB: carbapenem resistant *Acinetobacter baumannii*; *A. baumannii*; *Acinetobacter baumannii*; CSF: cerebrospinal fluid; CLSI: Clinical and Laboratory Standards Institute; PCR: polymerase chain reaction; MLST: multilocus sequence typing; STs: sequence types; CC: clonal complex; CHINET: China antimicrobial surveillance network; NDM: New Delhi metallo- $\beta$ -lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; ICU: intensive care unit; T: time; MIC: minimum inhibitory concentration; CSAB: carbapenem sensitive *Acinetobacter baumannii*; ISMRKP: imipenem sensitive but meropenem resistance *Klebsiella pneumoniae*; MALDI-TOF MS: matrix assisted laser desorption ionization time of flight mass spectrometry; NCBI: National Center for Biotechnology Information; T/C: ticarcillin/clavulanic acid; P/T: piperacillin/tazobactam; CAZ: ceftazidime; CSL: cefoperazone/sulbactam; FEP: cefepime; IPM: imipenem; MEM: meropenem; TOB: tobramycin; CIP: ciprofloxacin; LEV: levofloxacin; MNO: minocycline; TGC: tigecycline; COL: colistin; SXT: sulfamethoxazole/trimethoprim.

## Declarations

### Acknowledgements

Not applicable.

### Availability of data and materials

The datasets during and analyze during the current study available from the corresponding author on reasonable request.

### Authors' contributions

JZW conceived the idea and designed the experiment. JZW, XHS and HW analyzed the results. JZW, XHS and HW drafted the manuscript. RD, SQ, DYL, YFF and ZZH performed the experiment. XW, HQJ, LZ and BKS participated in manuscript revision. All authors read and approved the final manuscript.

XHS and HW contributed equally to this work.

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## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interests

Authors declare that they have no competing interests.

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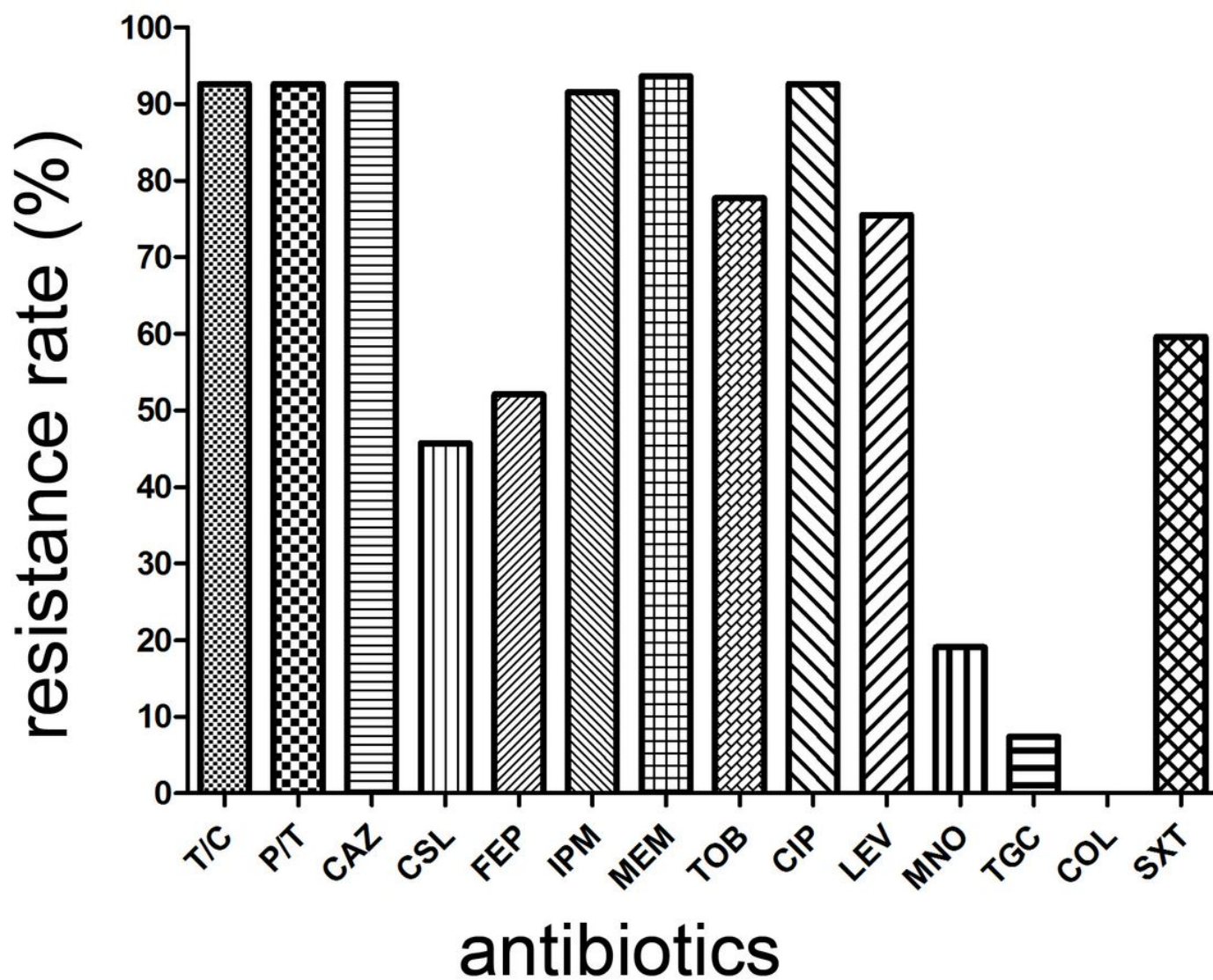
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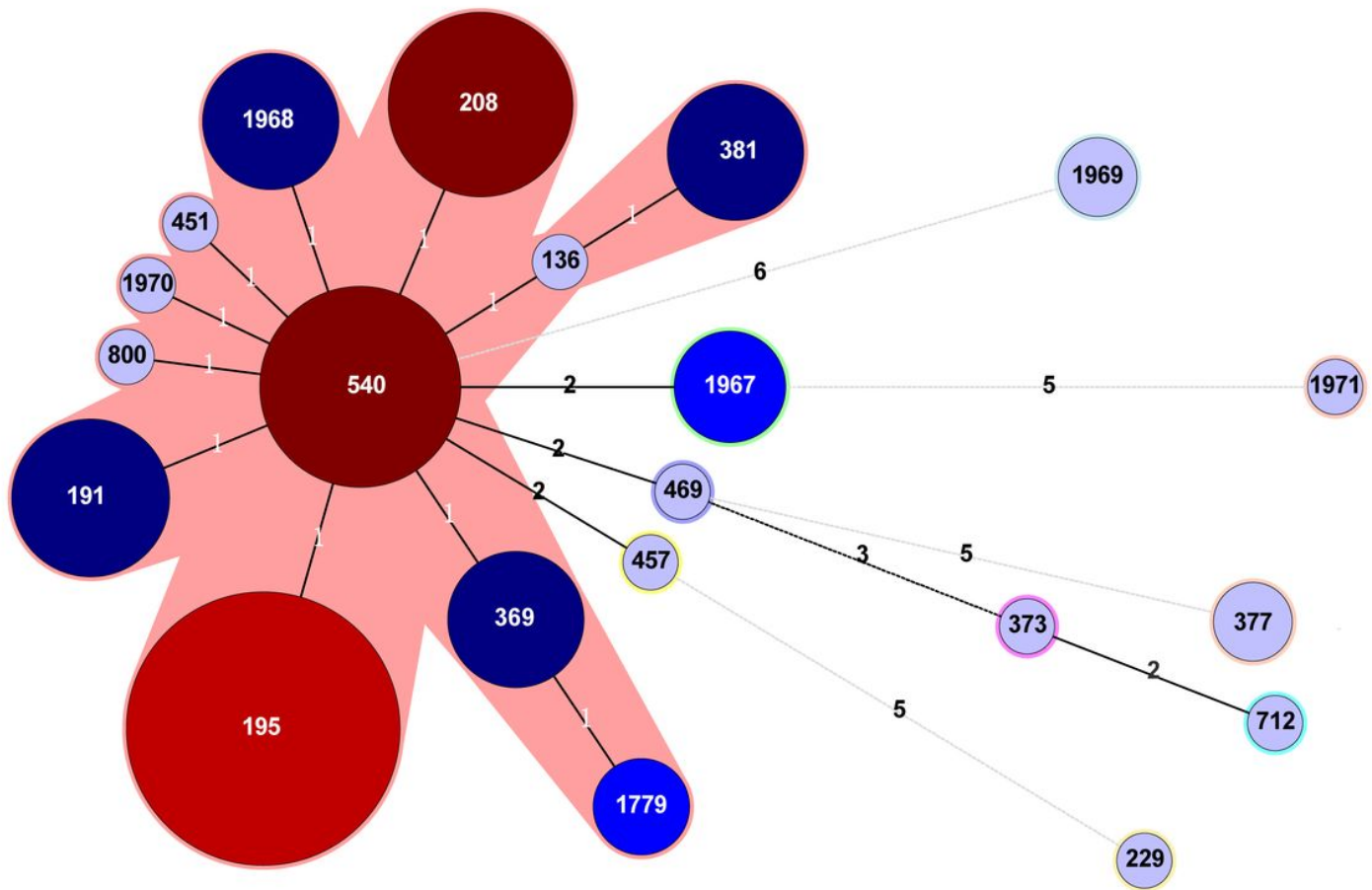
## Figures



**Figure 2**

Antibiotic resistance rates T/C: ticarcillin/clavulanic acid; P/T: piperacillin/tazobactam; CAZ: ceftazidime; CSL: cefoperazone/sulbactam; FEP: cefepime; IPM: imipenem; MEM: meropenem; TOB: tobramycin; CIP: ciprofloxacin; LEV: levofloxacin; MNO: minocycline; TGC: tigecycline; COL: colistin; SXT: sulfamethoxazole/trimethoprim.





**Figure 4**

Minimum spanning tree of 94 *Acinetobacter baumannii* isolates from cerebrospinal fluid and blood based on MLST. Each ST is represented by a circle sized in proportion to the number of isolates represented by that ST, the colors of the halo surrounding the STs denote types that belong to the same clonal complex, the number of allelic difference between STs is indicated on the branches. The detailed MLST profiles can be seen in Table S2 in Supplementary Material.

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