

A New OXA-66-harboring *Acinetobacter Baumannii* Sub-Clonal Complex 195 is Spreading Widely in Guangzhou, China, A Multicenter Study

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Short report

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Abstract

Objectives The clonal spread of *Acinetobacter baumannii* (*A. baumannii*) is an emerging problem. We analyzed the molecular epidemiology of *A. baumannii* isolated from 5 teaching hospitals in Guangzhou, China.

Methods 138 *A. baumannii* isolates were collected. Multilocus sequence typing (MLST) was used to assess the genetic relationships among the isolates. The *bla*_{OXA-51-like} gene was amplified and then sequenced.

Results Most of the isolates (55.8%, 77/138) were obtained from intensive care units (ICUs). The respiratory system was the most common site where *A. baumannii* was found (72.5%, 100/138). *A. baumannii* remained susceptible to polymyxin and tigecycline, but the susceptibility to other antimicrobial agents was below 30%. We used MLST to group the *A. baumannii* isolates into 8 existing sequence types (STs) and 17 new STs. With the predicted founder ST195 (accounted for 35.5% of all isolates, 49/138), Clonal complex (CC) 195 was the most prevalent and widely spread STs in the hospital environment. All ST195 isolates harbored OXA-66 according to the *bla*_{OXA-51-like} gene sequencing. But each hospital had its unique epidemiological feature.

Conclusions OXA-66 gene harboring CC195 was the most epidemic STs in Guangzhou, China. Health care facilities should develop their own management strategy.

Introduction

The clonal spread of multiple drug-resistant *A. baumannii* is an emerging problem worldwide[1–4]. *A. baumannii* is remarkable in its ability to acquire antibiotic resistance. It is also recognized as one of the most difficult health care-associated infections to control and treat[1, 5, 6]. Epidemiological studies and monitoring of the spread of *A. baumannii* may improve infection prevention and control. Previously, studies [7, 8]revealed CC92 represented the most epidemic sequence types (STs) in China. ST92 is the founder of CC92 and the predominant ST, while other STs belonging to CC92 vary by area. ST75 may be the most common epidemic ST in eastern China[9], whereas ST138 may be the most common ST in western China[10]. However, our study showed ST195 may be the major ST in Guangzhou and we did not detect any ST92 [11]. Another study also showed that ST195 and ST365 were the predominant STs from two hospitals in Guangzhou, China[12]. It seems ST195 but not ST92 may be more common in Guangzhou, China. However, these studies are limited by either single-center study or small sample size. Additionally, some studies have shown that sequence-based typing (SBT) of *bla*_{OXA-51-like} gene variants could be used to assess the epidemiological characterization of *A. baumannii*[13–15], but more data are needed. Therefore, a multicenter study is needed to better evaluate the issue. Here, we investigated 138 *A. baumannii* isolates from 5 teaching hospitals to determine the epidemic STs of the isolates in Guangzhou, the largest city of southern China.

Methods

Bacterial isolates and antimicrobial susceptibility testing

From April 2011 to April 2016, A total of 138 *A. baumannii* isolates were collected as part of the standard of care from 5 teaching hospitals (GFPH, Guangzhou First People's Hospital, the Second Affiliated Hospital of South China University of Technology, Pan Fu Road, Guangzhou, China; TAH, the Third Affiliated Hospital of Sun Yat-sen University, Tian He Road, Guangzhou, China; FAH, the First Affiliated Hospital of Sun Yat-sen University, Zhong Shan Er Road, Guangzhou, China; GXH, Guangzhou Xinhai Hospital, Xin Gang west Road, Guangzhou, China; GRCH, Guangzhou Red Cross Hospital, Tong Fu zhong Road, Guangzhou, China). Among the 138 *A. baumannii* isolates, 52 isolates had been reported in our previous study[5]. All *A. baumannii* isolates were obtained from clinical samples, such as bronchoalveolar lavage fluid, blood, sputum, cerebrospinal fluid, and urine. Duplicate isolates from the same patient were excluded.

The Vitek 2 (bioMerieux, Inc., Durham, NC, USA) automated microbiology system and AST-GN13 card were used for isolates identification and antimicrobial susceptibility tests. It included 13 antimicrobial agents (tigecycline, imipenem, amikacin, gentamycin, meropenem, piperacillin/tazobactam, ceftazidime, ceftriaxone, cefepime, aztreonam, levofloxacin, ciprofloxacin and trimethoprim-sulfamethoxazole). Disc diffusion method was used to detect the susceptibility of cefoperazone/sulbactam and polymyxin B. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI; M100-S22, 2012) [16]. Melone Pharmaceutical Co. Ltd. (China) provided antibiotic discs (OXOID). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control organisms.

Multilocus sequence typing and bla_{OXA-51-like} gene sequencing

According to Bartual et al. [17], MLST was performed as follows. 7 housekeeping genes, *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*, were amplified and then sequenced. The resulting sequences were assigned to STs using the Pubmlst database[18]. The eBURST algorithm (version 3) [19] was used to assess the genetic relationships by assigning STs to clonal complexes (CCs). CCs are defined as including any STs sharing alleles at ≥ 6 of 7 loci. The CC comprises a predicted founding ST as a common ancestor with other closely related STs descending from the predicted founding ST.

SBT-*bla*_{OXA-51-like} genes were carried out as follows. The *bla*_{OXA-51-like} genes were amplified and sequencing [20]. The sequences were analyzed using BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the genetic diversity of the *bla*_{OXA-51-like} genes[5, 15]. The primer sequences used in this study are listed in Supplementary table 1.

Results

Geographical distribution of the five teaching hospitals in Guangzhou City

5 teaching hospitals are from the central region of Guangzhou (Figure 1B). GFPH and FAH are located in the Yuexiu district; TAH is located in the Tianhe district; GXH and GRCH are located in the Haizhu district. Those three districts have more than 70% of the medical resources of Guangzhou[21] (Figure 1A).

Summary of 138 *A. baumannii* isolates

Among the 138 *A. baumannii* isolates, 66 isolates were from GFPH, 32 isolates were from FAH, 29 isolates were from GRCH, 6 isolates were from TAH and 5 isolates were from GXH (Table 1). Most of the patients (55.8%, 77/138), received care in intensive care units (ICUs), followed by the Emergency department (8.7%, 12/138), Department of Respiratory medicine (8.0%, 11/138) and Burn surgery department (5.8%, 8/138) and others (21.7%, 30/138) (Figure 2A and Table 1). However, each hospital had its unique pattern. Compared to other hospitals, the GFPH had more patients from Department of Respiratory medicine while FAH showed more patients in Neurology department. In GRCH, most patients came from Emergency department (34.5%, 10/29) (Figure 2A).

The respiratory system (72.5%, 100/138) was the most common site where *A. baumannii* was found, followed by the blood (14.5%, 20/138) and wounds (6.5%, 9/138). When comparing among hospitals, FAH had a higher portion of *A. baumannii* obtained from blood, while GFPH had more respiratory samples, and GRCH had more wound samples (Figure 2B).

Despite the clinical features of the *A. baumannii* isolates were vary by different hospitals, the antimicrobial susceptibility testing results were quite similar within the 5 hospitals. Among the 138 *A. baumannii* isolates, the susceptibility to polymyxin B was 99.1%, followed by tigecycline 89.8%, while its susceptibility to other antimicrobial agents was below 30% (Figure 3, supplementary table 2 and supplementary figure 1).

MLST and *SBT-bla_{OX}A-51-like* genes

By using MLST, 66 *A. baumannii* isolates from GFPH were grouped into 15 different STs, including 5 existing STs and 8 novel STs (STn1 to STn8). ST195 was the most common STs (33.3%, 22/66). The clonal relationship analysis showed ST195, ST208, ST136, ST457, ST533, ST548, STn1, STn2, STn5, STn7 and STn8 formed a clone complex (CC), named CC195. ST195 was predicted to be the founder of CC195 (Supplementary figure 2A and Table 1).

MLST grouped 32 *A. baumannii* isolates from FAH into 12 different STs, including 3 existing STs and 9 novel STs (STn2 and STn9 to STn16). The most common STs were ST195 and ST208 (62.5%, 20/32). The clonal relationship analysis demonstrated those isolates formed two CCs, one is CC195 with the

founder ST195 and the other CC including 2 new STs, STn9 and STn13 (Supplementary figure 2B). 29 *A. baumannii* isolates from GRCH were grouped into 9 different STs, including 6 existing STs and 3 novel STs (STn2, STn8 and STn17). ST195 was the most common ST (37.9%, 11/29). Those isolates formed a CC195 according to the clonal relationship analysis (Supplementary figure 2C and Table 1).

We analyzed 138 *A. baumannii* isolates from 5 hospitals. CC195, with its predicted founder ST195 (accounted for 35.5% of all isolates, 49/138), was the most epidemic STs (Figure 4A and Table 1). We combined 138 *A. baumannii* isolates with 225 *A. baumannii* isolates from MLST database (Accessed 22 Mar 2017) submitted by other researches from China and ran the clonal relationship analysis (Figure 4B, Table 1 and Supplementary table 3). The results showed CC92, with its founder ST92, was the most epidemic STs in China. Additionally, we also found a new sub-CC195 is forming in Guangzhou, China, and derived from CC92. (Figure 4B).

According to the *SBT-bla_{OXA-51-like}* genes, most *A. baumannii* isolates including all ST195 carried OXA-66 (95.7%, 132/138). Isolate_11 from GFPH carried OXA-199. Isolate_80, isolate_81, isolate_90 and isolate_98 from FAH carried OXA-68, OXA-121, OXA-441 and OXA-64 respectively. Isolate_80 and isolate_89 FAH carried a new *bla_{OXA-51-like}* gene named OXA-n1. OXA-n1 has a C-insertion mutation at 200 bases which is different from OXA-66 (EF534257.1) (Table 1).

Discussion

A. baumannii has emerged as one of the most troublesome pathogens in health care institutions globally. Due to antibiotic resistance and clonal spread, identifying potential reservoirs of the organism and modes of transmission to control the spread of *A. baumannii* are urgently needed. Currently, data from Guangzhou is very limited. To our knowledge, this study is the first multicenter epidemiological study regarding *A. baumannii* spreading in Guangzhou, the largest city in southern China. It provided an insight view of looking into the development of customized strategies for infection control.

The increasing resistance of *A. baumannii* to many antimicrobial agents in China has been noticed by the CHINET surveillance system. Especially, resistance to imipenem and meropenem has rapidly rising trend from 32.9% in 2005 to 77.1% in 2018 and from 41.3% in 2005 to 78.1% in 2018, respectively [22, 23]. According to our data, things are getting worse in Guangzhou, more than 80% of *A. baumannii* isolates showed resistance to imipenem and meropenem, and only tigecycline and polymyxin remained effective. However, tigecycline and polymyxin resistance *A. baumannii* has been reported and the resistance rate kept rising [24–27]. Therefore, monitoring and controlling the spread of *A. baumannii* is urgent.

Our study revealed CC195, with the predicted founder ST195, which was the most common CC identified within the 5 hospitals. It suggested the current epidemic strains in Guangzhou, China is CC195. Previously, studies found CC92 was the most epidemic STs in multiple provinces in China [7, 8]. Interestingly, ST92 is the predicted founder of CC92, and ST195 is the single locus variant of ST92 (Fig. 4B). However, we did not detect any ST92 in our previous report [11] or this study with larger

sample size. ST92 was not detected in other studies from China [28] or South Africa [29] either. Additionally, studies showed ST195, ST208, ST365, and ST191 (but not ST92) are the most common STs in hospitals across China [11, 12, 28]. One explanation for the absence of ST92 was ST195 evolved from ST92 and obtained more adaptation capacity to the hospital environment. With this evolution, ST195 may gradually replace ST92 and became the dominant clone (Fig. 4B). In our study, most of *A. baumannii* isolates including all ST195 carried OXA-66, which was similar to the previous report that 18 representative isolates from China carried the same OXA-66 allele [30]. This finding indicated OXA-66 gene harboring CC195 had strong adaptability to the environment and served as a new epidemic marker in Guangzhou City, China.

Our study revealed CC195 was spreading in the hospital. But each hospital had its unique epidemiological feature. Most patients of GRCH were from Emergency department while FAH had more patients in neurology department. This finding may reveal potential different reservoirs for CC195 within hospital settings. It suggests each hospital should develop its own management strategy.

Conclusion

OXA-66 gene harboring CC195 was the most epidemic STs in Guangzhou, China, and serves as a new epidemic marker. Health care facilities should develop their own management strategy.

Declarations

Ethics approval and consent to participate

The Guangzhou First People's Hospital, School of Medicine, South China University of Technology Ethics Committee approved the study. All the patients were provided informed consent for inclusion in the study.

Consent for publication

All authors read and approved the final manuscript for publication

Availability of data and material

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YjL supervised the study, performed the susceptibility testing and MLST, and wrote the manuscript. XmH, WyY and CzP discussed the data and helped finalize the manuscript. ZxM, McH and CqF contributed to the susceptibility testing and sequence-based typing of the *bla*_{OXA-51-like} genes. HIC and PhG provided advice regarding the susceptibility testing technology. ZwZ and SqW planned and supervised the experiments.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

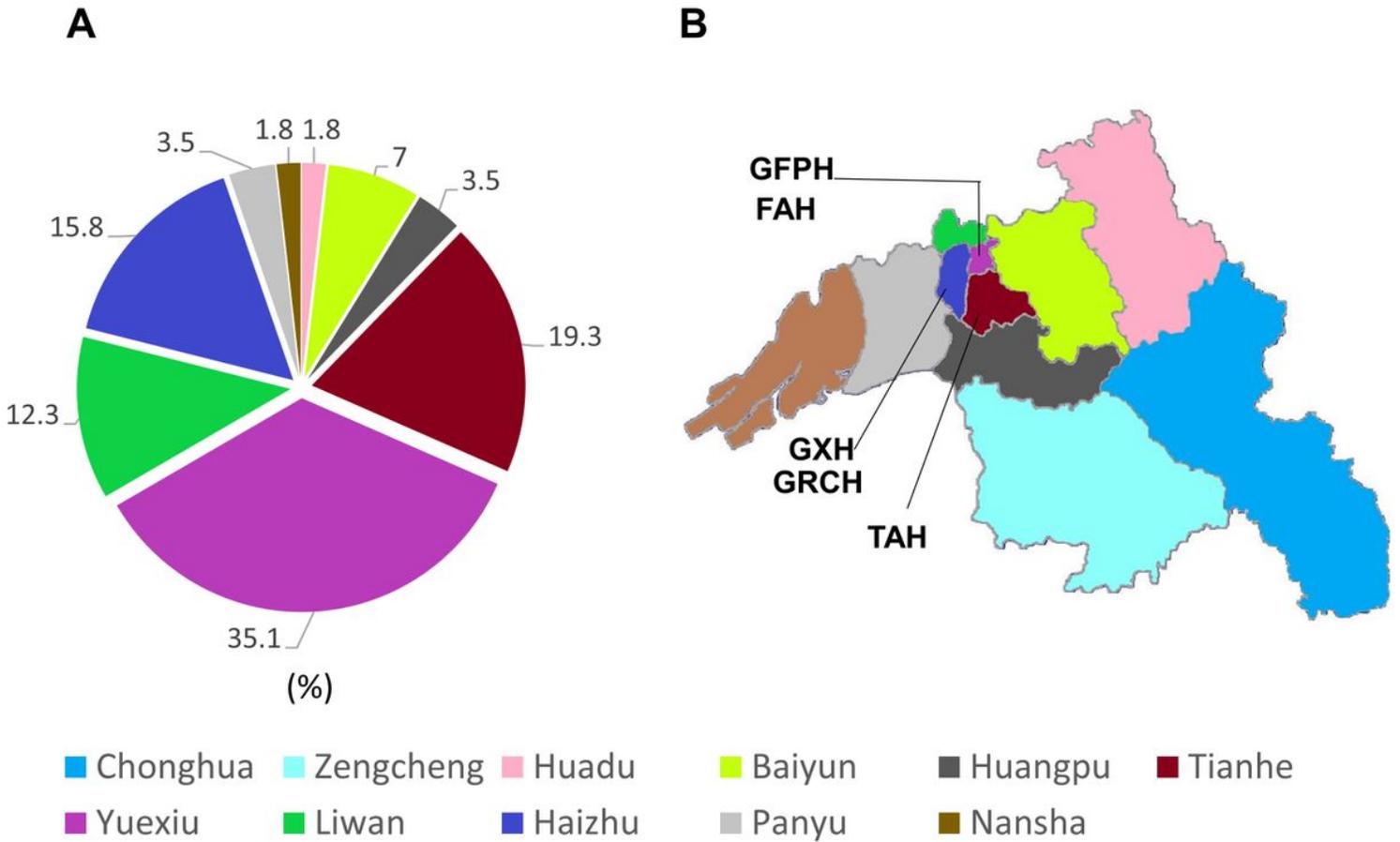


Figure 1

Geographical distribution of the five teaching hospitals in Guangzhou City. There are 11 districts named and marked by different color in Guangzhou city; A, percentage of three grade A teaching hospital within each district; B, geographical distribution of the five teaching hospitals on the map of Guangzhou city; GFPH, Guangzhou First People's Hospital; FAH, First Affiliated Hospital of Sun Yat-sen University; TAH, Third Affiliated Hospital of Sun Yat-sen University; GXH, Guangzhou Xinhai Hospital; GRCH, Guangzhou Red Cross Hospital.

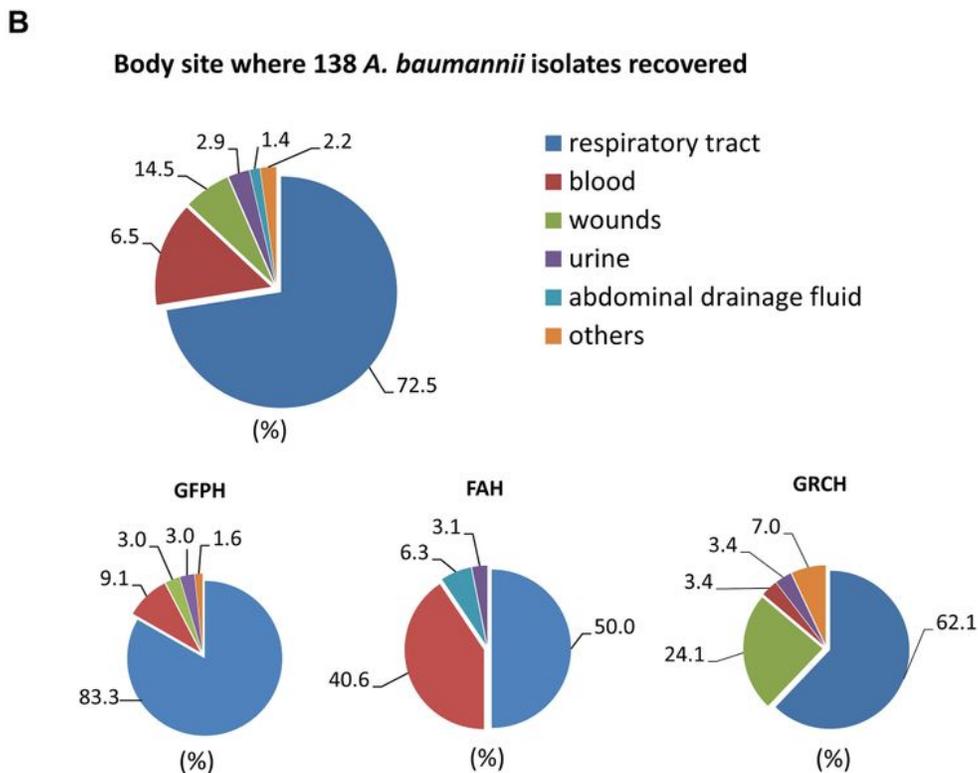
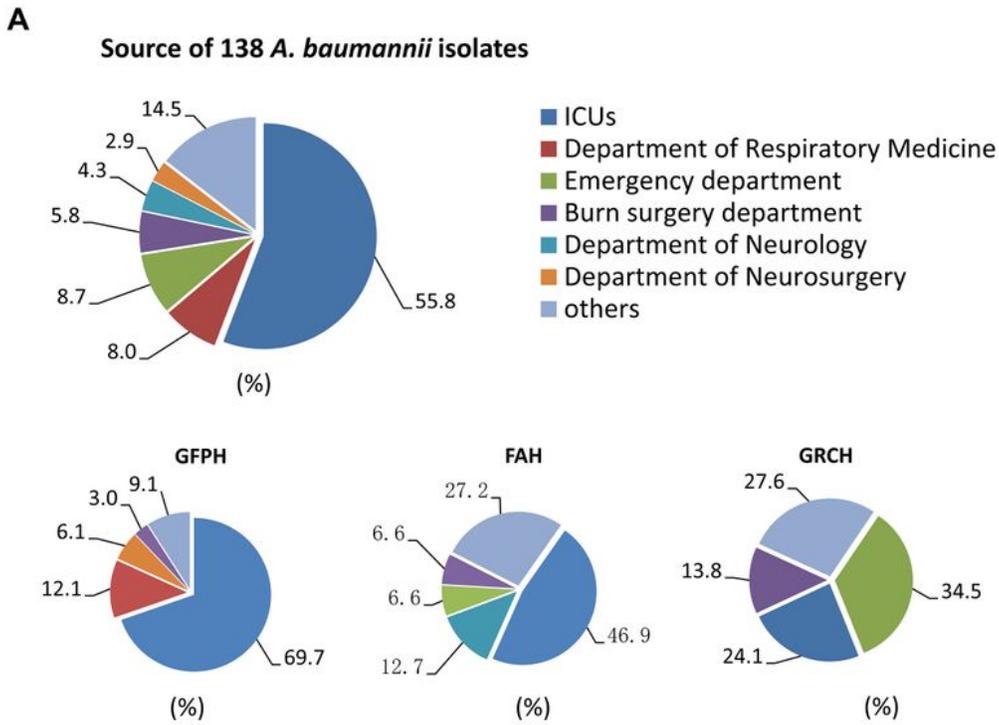


Figure 2

Characteristics of the 138 *A. baumannii* isolates. A, percentage of *A. baumannii* isolates from different sources of hospital setting; B, percentage of *A. baumannii* isolates from different body site of patient; ICUs, Intensive care units; GFPH, Guangzhou First People's Hospital; FAH, First Affiliated Hospital of Sun Yat-sen University; GRCH, Guangzhou Red Cross Hospital.

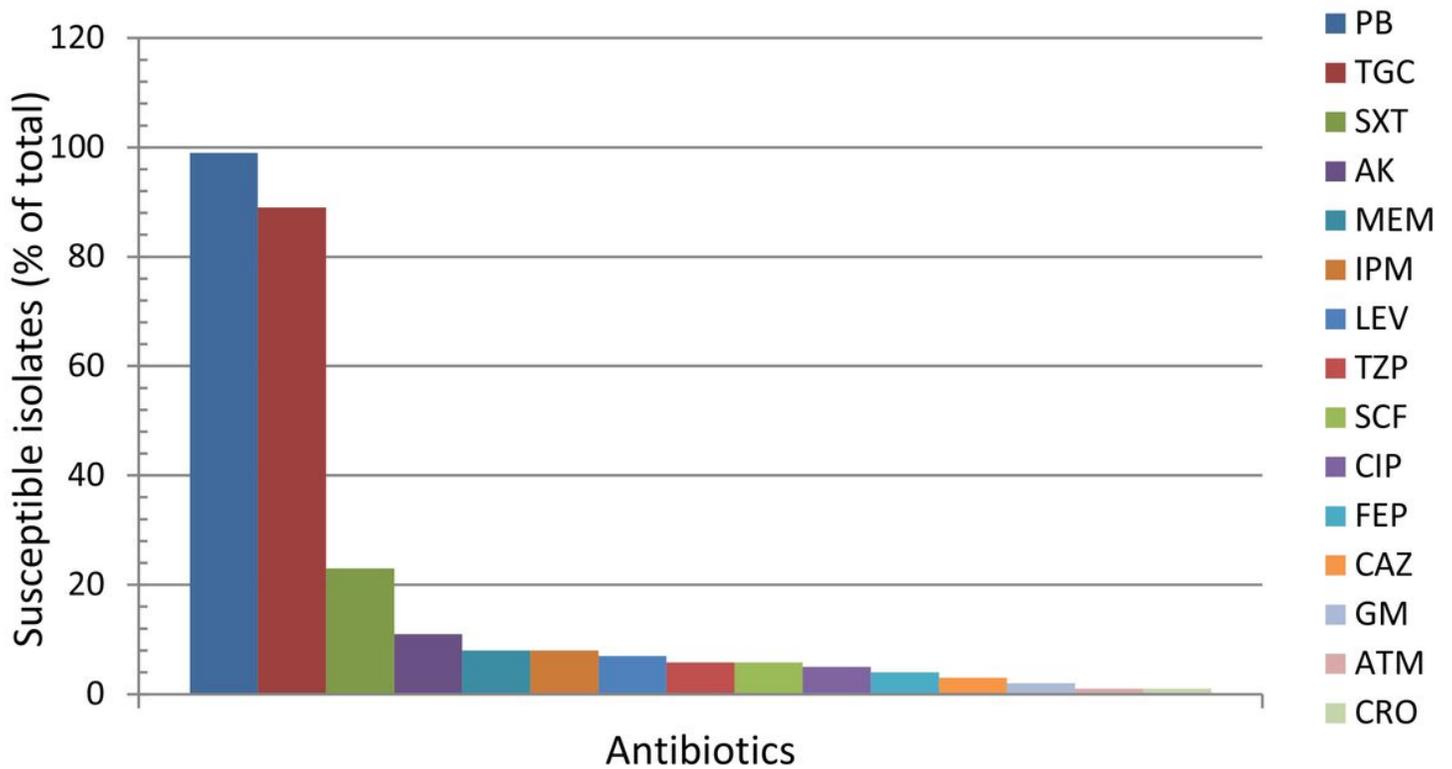


Figure 3

Antimicrobial susceptibility testing of the 138 *A. baumannii* isolates. PB, polymyxin B; TGC, tigecycline; IPM, imipenem; AK, amikacin; GM, gentamycin; MEM, meropenem; TZP, piperacillin/tazobactam; SCF, cefoperazone/sulbactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; LEV, levofloxacin; CIP, ciprofloxacin; SXT, Trimethoprim-sulfamethoxazole.

indicates CC195. The ST labels are coloured as follows: black, STs found only in the Pubmlst database; green, STs found only in this study; and purple, STs found in both the Pubmlst database and this study.

Supplementary Files

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