Wide dissemination of OXA-23-producing carbapenem-resistant Acinetobacter baumannii clonal complex 22 in multiple cities of China

Yiqi Fu¹, Jianying Zhou¹, Hua Zhou¹, Qing Yang², Zeqing Wei², Yunsong Yu²* and Lanjuan Li²

¹Department of Respiratory Diseases, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, People’s Republic of China; ²State Key Laboratory for Diagnosis and Treatment of Infectious Disease, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, People’s Republic of China

*Corresponding author. Tel: +86 571 8723 6421; Fax: +86 571 8723 6423; E-mail: yvys119@163.com

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Objectives: In this study, multilocus sequence typing (MLST) was used to describe the genetic backgrounds of carbapenem-resistant Acinetobacter baumannii (CRAB) and carbapenem-susceptible A. baumannii (CSAB) from multiple cities of China.

Methods: One hundred and fifty-two CRAB and 74 CSAB isolates obtained from 16 cities of China were selected for molecular characterization by MLST. eBURST was used to cluster sequence types (STs) into clonal complex (CCs) and infer evolutionary descent. PCR was used to detect carbapenemase-encoding genes and blaAmpC with the upstream element ISAba1.

Results: CSAB showed more diverse genetic backgrounds than CRAB since 36 distinct STs were identified in CSAB while only 8 STs were identified in CRAB. ST22 and its three single-locus variants, all clustered into CC22, were the most prominent STs, accounting for 86.8% of CRAB and 45.9% of CSAB, distributed in all 16 cities and possessing more noticeable antibiotic resistance than other STs. PCR amplification was positive for blaOXA-23 in most CRAB isolates but negative in CSAB isolates. The presence of ISAba1 upstream of blaAmpC was variable in distinct STs of CRAB. eBURST reveals that CC22 is the largest group in the Pubmlst database, which also contains ST6 previously identified in a European clone II isolate as a member of a subgroup of CC22.

Conclusions: We describe the wide dissemination of CRAB CC22 in China. The close relatedness between CC22 and European clone II implies the probable global spread of CC22. It is inferred that ST22-CSAB evolves to ST22-CRAB through acquiring blaOXA-23 as a determinative factor.

Keywords: MLST, clonal dissemination, European clone II

Introduction

Acinetobacter baumannii is notorious for its remarkable ability to acquire antibiotic resistance and cause persistent nosocomial infections. Due to the wide application of broad-spectrum antibiotics, the resistance rates of A. baumannii to most antibiotics have continually increased during recent decades; resistance to carbapenems is most concerning. The MYSTIC programme of 2007 demonstrated that 74.1% of isolates were susceptible to meropenem and 78.9% were susceptible to imipenem in Europe, compared with much lower susceptibilities of 51.3% and 52.0% in several Asian countries in the SENTRY programme of 2006–07. The emergence of carbapenem-resistant A. baumannii (CRAB) has been described as the sentinel event of antimicrobial resistance.

Molecular characterization reveals that clonal dissemination plays an important role in nosocomial outbreaks of CRAB, and some epidemic clones spread nationwide and even cross-nation, e.g. several cross-China major CRAB clones, genetically related OXA-23-producing clones in several Asian countries, OXA-23 clones 1 and 2 in hospitals in London and south-east England, and European clone II, reflecting that certain resistant strains have advantages in surviving in a hospital environment and causing outbreaks of nosocomial infections. However, most studies carried out molecular genotyping by PFGE, which is criticized for its poor ability to compare results between laboratories and for being overdiscriminatory for large-scale or long-term molecular epidemiological investigations. Therefore, less is known about the link between these epidemic clones and it is an urgent task to describe the relatedness of the
epidemic CRAB clones distributed in distinct regions of the world and to validate the worldwide dissemination of CRAB clones by other typing methods. Moreover, few studies focused on the difference in genetic backgrounds between carbapenem-susceptible A. baumannii (CSAB) and CRAB. It is also unclear whether a certain genetic background is related to acquiring antibiotic resistance or evolving from CSAB to CRAB.

Multilocus sequence typing (MLST) provides an unambiguous typing method by identifying accurate and portable nucleotide sequences of internal fragments of multiple-loci housekeeping genes, which has achieved notable success in global epidemiological investigation of Staphylococcus aureus and Neisseria meningitidis and Enterococcus faecium, as well as in the evolutionary history of methicillin-resistant S. aureus (MRSA) and carbapenem-resistant A. baumannii ST22 in intensive care units of a Seoul hospital, but less discussion has been focused on the evolution of CRAB.

We previously reported clonal dissemination of CRAB harbouring blaoXA-23 among different cities in China based on the analysis of PFGE patterns. In this study, MLST was applied for further characterization of the genetic diversity of these CRAB isolates. Meanwhile, some CSAB isolates were taken into consideration to compare the genetic diversity with that of CRAB and to explore the evolutionary descent of CRAB.

Methods

Bacterial isolates and species identification

A total of 226 non-duplicated clinical A. baumannii isolates collected from multiple cities of China from January 2005 to December 2005 were selected for analysis according to the distribution of regions and pulsotypes. One hundred and fifty-two CRAB isolates from 24 tertiary hospitals of 16 cities [Beijing (5 isolates), Chongqing (4 isolates), Shanghai (13 isolates), Guangzhou (6 isolates), Shenyang (5 isolates), Hangzhou (47 isolates), Huzhou (8 isolates), Jinhu (8 isolates), Jiaxing (5 isolates), Shaoxing (7 isolates), Ningbo (6 isolates), Taizhou (7 isolates), Wenzhou (6 isolates), Quzhou (8 isolates) and Zhoushan (1 isolate)] represented previously identified epidemic PFGE clone A (27 isolates), clone B (17 isolates), clone C (77 isolates), clone D (15 isolates), clone E (4 isolates) and sporadic genotypes (12 isolates). Beijing, Shanghai, Guangzhou, Chongqing, Shenyang and Hangzhou are major cities located in different parts of China. Hangzhou, Huzhou, Jinhu, Jiaxing, Shaoxing, Taizhou, Wenzhou, Quzhou and Zhoushan are all part of Zhejiang Province in eastern China (Figure 1). All isolates have an imipenem and/or meropenem MIC $>16$ mg/L.

Seventy-four CSAB (imipenem and meropenem MICs both $<=4$ mg/L) were obtained during the same period from Beijing (12 isolates), Chongqing (8 isolates), Hangzhou (3 isolates), Jiaxing (9 isolates), Shaoxing (1 isolate), Taizhou (6 isolates), Wenzhou (7 isolates) and Zhoushan (7 isolates).

Species identification was performed by sequence analysis of the 16S–23S rRNA gene spacer region and the intrinsic blaoXA-51-like gene as previously described.

MLST

MLST was carried out as described by Bartual et al. for all isolates. In brief, internal fragments of seven housekeeping genes, i.e. gltA, gyrB, gdhB, recA, cpn60, gpi and rpoD, were PCR amplified, followed by purification and then sequencing with an ABI prism sequencer 3730 (Applied Biosystems, USA). In order to obtain specific PCR products and satisfactory sequencing results, three primer pairs were redesigned: gyrB_up, GAATGCTGGTGTACGTATCG and gyrB_dw, ACGCTCAACGTTCAGGATCT (for both amplification and sequencing); rpoD_P1, CTAGGCTGTTAGTACGTTAG and rpoD_dw, ACGGCTAAGTGTCAGCT (for sequencing). However, the internal fragments for analysis were identical to a previous scheme. BLAST was run to

Figure 1. Location of the 16 cities in this study and the distribution of four major CRAB STs in China. Filled circles, ST22; filled squares, STn1; open circles, STn2; open squares, STn3.
compare the sequence of each allele with existing sequences in the Pubmlst database (http://pubmlst.org/abaumannii/). STs were designated according to the allelic profiles. A-G represented the seven housekeeping genes in the order gisA, gyrB, gdhB, recA, cpn60, gpi and rpoD, respectively, and the novel unassigned alleles were designated 1, 2, 3, etc. consecutively. The sequences of novel alleles are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/). eBURST (version 3, http://eburst.mlst.net/) was used to assign STs to clonal complexes (CCs) and assess the genetic relatedness of STs with the most stringent definition of the groups by sharing alleles at ≥6 of 7 loci.\(^\text{25}\) A CC comprises a founding ST as a common ancestor and several other closely related STs descended from the predicted founding genotype.\(^\text{24}\) The default computation of bootstrap values (1000 re-sampling) was used to estimate the confidence of the predicted founder of a CC.\(^\text{24}\)

**Antimicrobial susceptibility testing**

Susceptibility of the isolates to ceftazidime, ceftriaxone, ciprofloxacin, cefotaxime, amikacin, ciprofloxacin, minocycline, ampicillin/sulbactam and piperacillin/tazobactam was determined by the disc diffusion method. The MICs of imipenem and meropenem were determined by the agar dilution method, and the MIC of colistin was determined by Etest (AB bioMerieux, France). Interpretation was in accordance with the CLSI 2009 standard.\(^\text{25}\)

**Detection of carbapenemase-encoding genes and bla\textsubscript{AmpC}**

Our previous study indicated that \textbf{bla}\textsubscript{OXA-23} and \textbf{bla}\textsubscript{OXA-51} with or without the presence of an IS\textsubscript{aba1} insertion upstream were the most common carbapenemase-encoding genes contributing to carbapenem resistance of A. baumannii in China, and \textbf{bla}\textsubscript{OXA-24-ike}, \textbf{bla}\textsubscript{OXA-58-ike}, \textbf{bla}\textsubscript{IMP}, \textbf{bla}\textsubscript{VIM} and \textbf{bla}\textsubscript{OXA-48} genes were not detected in our collected CRAB isolates.\(^\text{7}\) Therefore, these genes were detected by using PCR with the same primers for CSAB isolates. PCR assays were performed using primers for the intrinsic cephalosporinase gene (\textbf{bla}\textsubscript{AmpC}) and upstream IS\textsubscript{aba1} as described previously.\(^\text{26}\)

**Statistical analysis**

Resistance rates for each antibiotic were compared between CRAB and CSAB, CC22 and other STs, STn3 and other STs, and ST22-CSAB and ST22-CRAB by using Fisher’s exact test. A P value <0.05 was considered to indicate a statistically significant difference.

**Results**

**MLST of CRAB**

The molecular characterization of 152 CRAB by MLST identified eight distinct STs. ST22 (corresponding to PFGE clone A, partial clone C, clone E and partial sporadic clones) and its two novel single-locus variants (SLVs) (STn1, corresponding to partial clone C, and STn2, corresponding to clone B) were the predominant STs, comprising 55.9%, 19.7% and 11.2%, respectively. ST22 was also the most widely disseminated ST, which was identified in isolates from as many as 14 cities across China; STn1 and STn2 were less widely disseminated (five cities and three cities, respectively). A novel double-locus variant of ST20 (STn3, corresponding to clone D) was another major ST recovered from four tertiary hospitals in two cities in Zhejiang Province, accounting for 9.9% of isolates. The remaining STs comprised ST20 (two isolates, Huzhou, corresponding to sporadic clones), STn5, STn6 and STn7 (one isolate each, corresponding to sporadic clones) from Beijing, Shanghai and Taizhou, respectively (Table 1).

**MLST of CSAB**

A total of 36 different STs were identified in 74 CSAB. Similar to the distribution of STs in CRAB, ST22 also achieved a dominant position in CSAB, accounting for 43.2% isolates from 11 cities (Table 1). A novel SLV of ST22 (STn4) was identified in two isolates from Zhourshan (Table 1). In addition to ST22, ST20 was another ST identified in both CRAB and CSAB, despite there being only two isolates in each group (Table 1), and STn27 as an SLV of ST20 was identified in a CSAB isolate from Shanghai. Among the remaining 37 CSAB isolates, 32 STs including 29 novel STs were found, and each ST had no more than two isolates, except for ST17 (three isolates). With the exception of ST22 and ST20, none of the STs identified in CRAB isolates was also found in CSAB isolates. Isolate information and the allelic profiles of CSAB are listed in Table S1 [available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)] (except for ST22-CSAB and ST20-CSAB).

**Genetic lineage analysis by eBURST**

Up to 21 October 2009, based on the data contained in the Pubmlst database (http://pubmlst.org/abaumannii/), the 42 STs identified in this study were clustered into two CCs. ST22, STn1, STn2 and STn4 belonged to CC22 with ST22 as the predicted founder and previously assigned ST53 as the founder of a sub-group of CC22. However, a relatively low ST bootstrap value of 70% suggested a relatively poor confidence in the predicted founder ST22. Meanwhile, a similar ST bootstrap value (65%) estimating the confidence in ST53 as the founding ST made it difficult to confirm the real founder of this CC. When a population snapshot was performed for all STs, ST53 was assigned as the founding ST since it had more triple-locus variants than ST22. However, according to the Pubmlst database, ST22 has been recovered from Italy, Korea, China Hong Kong, Australia, Portugal and the Czech Republic (http://pubmlst.org/abaumannii/), suggesting that it is the most predominant ST, which provides additional evidence to support it as the founding ST.\(^\text{24}\) ST25 identified in a CSAB isolate belonged to CC44. STn27 is an SLV of ST20, implying a probable relatedness to each other. In addition, two pairs of novel STs (STn11 and STn13, and STn17 and STn23) identified in CSAB isolates were clustered into two groups. The remaining STs all fell into distinct singletons; 4 singletons in CRAB and 27 singletons in CSAB (Figure 2).

**Antibiotic resistance profiles**

In general, the CRAB isolates were more frequently resistant to other antibiotics than the CSAB isolates (Table 2). When compared among different STs, it was apparent that isolates of CC22 and STn3 showed more significant resistance to antibiotics than other STs (Table 2). All STn3 isolates had imipenem MIC\textsubscript{C}\textsubscript{95}> 32 mg/L and 90.36% of CC22 isolates had imipenem MIC\textsubscript{C}\textsubscript{95}> 4 mg/L, while the percentage was 11.1% for isolates of other STs. The meropenem data were similar to those for imipenem (data not shown). Noticeably, there were no statistically significant differences in the resistance rates for other antibiotics between ST22-CSAB and ST22-CRAB (Table 2). In addition, the
Table 1. Distribution, imipenem and meropenem MICs and allelic profiles of CRAB and related CSAB

<table>
<thead>
<tr>
<th>STa</th>
<th>Allelic profilesb</th>
<th>Distribution of cities (no. of isolates)c</th>
<th>IPM/MEM MIC</th>
<th>CRAB</th>
<th>IPM/MEM MIC</th>
<th>CSAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no.</td>
<td>range (mg/L)d</td>
<td>no.</td>
</tr>
<tr>
<td>ST22-blaOXA-23(+)</td>
<td>1-3-3-2-2-7-3</td>
<td>BJ (1), CQ (1), HZ (17), GZ (5), HUZ (6), JH (5), NB (7), SH (5), SX (16), TZ (4), WZ (4), QZ (1)</td>
<td>72</td>
<td>32-128/32-128</td>
<td>0</td>
<td>32-128/32-128</td>
</tr>
<tr>
<td>ST22-blaOXA-23(−)</td>
<td>1-3-3-2-2-7-3</td>
<td>BJ (37), CQ (3/4), HZ (9/2), GZ (1/0), JX (1/0), LS (9/1), NB (1/4), SH (1/2), SX (1/1), TZ (1/2), WZ (2/2), ZS (1/4)</td>
<td>13</td>
<td>4-32/8-32</td>
<td>32</td>
<td>1-4/1-4</td>
</tr>
<tr>
<td>STn1-blaOXA-23(+)</td>
<td>1-3-3-2-2-6-2</td>
<td>HZ (13), JX (3), LS (8), TZ (1), QZ (5)</td>
<td>30</td>
<td>32-168/64-128</td>
<td>0</td>
<td>32-128/32-128</td>
</tr>
<tr>
<td>STn2-blaOXA-23(+)</td>
<td>1-3-3-2-2-11-3</td>
<td>HZ (5), SH (7), SY (5)</td>
<td>17</td>
<td>32-128/32-128</td>
<td>0</td>
<td>32-128/32-128</td>
</tr>
<tr>
<td>STn3-blaOXA-23(+)</td>
<td>1-2-15-13-12-4-6-2</td>
<td>HZ (12), JH (3)</td>
<td>15</td>
<td>32</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>STn4-blaOXA-23(−)</td>
<td>1-3-3-2-2-10-3</td>
<td>ZS (2)</td>
<td>0</td>
<td>32/16</td>
<td>2</td>
<td>2/1</td>
</tr>
<tr>
<td>STn5-blaOXA-23(−)</td>
<td>A1-12-40-26-E1-F1-5</td>
<td>BJ (1)</td>
<td>1</td>
<td>32/16</td>
<td>0</td>
<td>32/16</td>
</tr>
<tr>
<td>STn6-blaOXA-23(−)</td>
<td>1-34-C1-28-E2-F1-G1</td>
<td>SH (1)</td>
<td>1</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>STn7-blaOXA-23(+)</td>
<td>1-B1-C2-6-1-F2-G2</td>
<td>TZ (1)</td>
<td>1</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>ST20-blaOXA-23(−)</td>
<td>1-15-13-12-4-12-2</td>
<td>HUZ (2)</td>
<td>2</td>
<td>128/128</td>
<td>0</td>
<td>2/1</td>
</tr>
<tr>
<td>ST20-blaOXA-23(−)</td>
<td>1-15-13-12-4-12-2</td>
<td>LS (1), HZ (1)</td>
<td>0</td>
<td>128/128</td>
<td>0</td>
<td>128/128</td>
</tr>
</tbody>
</table>

aST, sequence type. The novel unassigned STs were designated STn1, n2, n3, etc. consecutively.
bSeven loci in the order gltA, gyrB, gdhB, recA, cpn60, gpi, rpoD. A–G represented seven housekeeping genes, respectively, and the novel unassigned alleles were designated 1, 2, 3, etc. consecutively.

cAbbreviations of cities: BJ, Beijing; CQ, Chongqing; SH, Shanghai; GZ, Guangzhou; SY, Shenyang; HZ, Hangzhou; HUZ, Huzhou; JH, Jinhua; JX, Jiaxing; LS, Lishui; NB, Ningbo; SX, Shaoxing; TZ, Taizhou; WZ, Wenzhou; QZ, Quzhou; ZS, Zhoushan.

dIPM, imipenem; MEM, meropenem.
eNumber of CRAB isolates/number of CSAB isolates.

Distribution of blaOXA-type genes and blaAmpC

The blaOXA-type gene was not detected in any of the CSAB isolates. In contrast, the blaOXA-type gene was identified in the majority of CRAB isolates, including all isolates of ST22, ST25 and ST3. The ST22-CRAB isolates contained 100% of the isolates harbouring this gene, except for two isolates of ST22. For 13 isolates of ST22-CRAB, the MIC ranges of imipenem and meropenem were 4–32 mg/L and 8–32 mg/L, respectively. In the remaining isolates, the MIC of imipenem was 128 times higher than those of CRAB. The positive rates were 96.5%, 81.3%, and 79.6% for CSAB of STn1-CRAB, STn2-CRAB, and STn3-CRAB, respectively.

Discussion

In the present study, MLST was used to evaluate the population genetic background of A. baumannii in Chinese hospitals. The analysis revealed that the ST22-CRAB isolates clustered into many more singletons than the STs of CSAB, which confirmed that the genetic backgrounds of the STs of CRAB are more diverse than those of CSAB. The eBURST algorithm revealed that the eBURST algorithm revealed that the eBURST algorithm revealed that the genetic backgrounds of the STs of CSAB were more diverse than those of CRAB. The positive rates were 96.5%, 81.3%, and 79.6% for CSAB of STn1-CRAB, STn2-CRAB, and STn3-CRAB, respectively. The positive rates were 96.5%, 81.3%, and 79.6% for CSAB of STn1-CRAB, STn2-CRAB, and STn3-CRAB, respectively.

The blaOXA-type gene was not detected in any of the CSAB isolates. In contrast, the blaOXA-type gene was identified in almost all of the isolates of ST22, ST25 and ST3. The ST22-CRAB isolates contained 100% of the isolates harbouring this gene, except for two isolates of ST22. For 13 isolates of ST22-CRAB, the MIC ranges of imipenem and meropenem were 4–32 mg/L and 8–32 mg/L, respectively. In the remaining isolates, the MIC of imipenem was 128 times higher than those of CRAB. The positive rates were 96.5%, 81.3%, and 79.6% for CSAB of STn1-CRAB, STn2-CRAB, and STn3-CRAB, respectively.

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Figure 2. Population snapshot of *A. baumannii* derived from this study and existing isolates in the Pubmlst database by eBURST algorithm. The radial diagram reflects the predicted evolutionary descent from the founder ST. A circle represents an ST, and its size corresponds to the number of isolates. Number indicated by single underlining represent STs only identified in this study, and numbers indicated by double underlining represent STs identified in both the present study and previously. The broken line indicates CC22.

Table 2. Other antibiotic resistance rates of *A. baumannii*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistance rate (%)</th>
<th>Resistance rate (%)</th>
<th>Resistance rate (%)</th>
<th>Resistance rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRAB</td>
<td>CSAB</td>
<td><em>P</em> value</td>
<td>CC22</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>94.7</td>
<td>56.8</td>
<td>&lt;0.001</td>
<td>100</td>
</tr>
<tr>
<td>Ceferpine</td>
<td>98.0</td>
<td>56.8</td>
<td>&lt;0.001</td>
<td>97.6</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>96.7</td>
<td>60.8</td>
<td>&lt;0.001</td>
<td>98.2</td>
</tr>
<tr>
<td>Amikacin</td>
<td>91.4</td>
<td>51.4</td>
<td>&lt;0.001</td>
<td>92.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>98.0</td>
<td>56.8</td>
<td>&lt;0.001</td>
<td>99.4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>98.0</td>
<td>58.1</td>
<td>&lt;0.001</td>
<td>99.4</td>
</tr>
<tr>
<td>Minocycline</td>
<td>87.5</td>
<td>45.9</td>
<td>&lt;0.001</td>
<td>88.6</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>98.0</td>
<td>59.5</td>
<td>&lt;0.001</td>
<td>98.2</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>70.4</td>
<td>37.8</td>
<td>&lt;0.001</td>
<td>72.3</td>
</tr>
<tr>
<td>Colistin</td>
<td>13.8</td>
<td>1.4</td>
<td>&lt;0.001</td>
<td>12.0</td>
</tr>
</tbody>
</table>

* Differences between antibiotic resistance rates were compared between CC22 and other STs, and between STn3 and other STs.
that CC22 represented the most epidemic STs in China, accounting for 86.8% of CRAB and 45.9% of CSAB isolates presented in this study. It is also the principal CC in the existing database including several previously identified STs. Interestingly, ST6, corresponding to a European clone II strain, was an SLV of a subgroup of CC22 (Figure 2). European clone II is known as a widespread multidrug-resistant A. baumannii clone and has been found in Spain, Portugal, South Africa, France, Greece, Turkey and the Czech Republic. Recently, the emergence of CRAB belonging to European clone II has been reported in the Czech Republic, Italy and Germany. Moreover, the occurrence of multidrug-resistant A. baumannii genetically related to European clone II has been described in the US Walter Reed Army Medical Center and another military treatment facility. Our study revealed the probable relatedness between European clone II and CRAB prevalent in China. In addition, researchers from Italy, South Korea, China Hong Kong, Australia, Portugal and the Czech Republic have also submitted ST22 to the Pubmlst database. In addition to A. baumannii ST22 producing OXA-23 from South Korea being resistant to carbapenems,15,20 ST22 from Australia also showed resistance to meropenem (http://pubmlst.org/abaumannii), validating the existence of a globally epidemic clone of A. baumannii. It has been proved that the outbreak of multidrug-resistant A. baumannii in US military hospitals was largely attributed to injured soldiers colonized or infected with A. baumannii returning from Iraq, which provides the probability of cross-continent transmission of A. baumannii. However, the existence of a globally disseminated ST implies another explanation that a special genetic background with advantages in acquiring antibiotic resistance and surviving in a hospital environment make it successfully selected under antibiotic pressure.

Prominent antibiotic resistance is a special feature of CC22. ST22 and its SLVs identified in this study are all multidrug-resistant isolates, and 14 isolates of CC22 even showed resistance to all tested antibiotics, including colistin. Recently, researchers in South Korea reported the emergence of XDR A. baumannii ST22, which was resistant to all antimicrobial agents including tigecycline, polymyxin B and colistin.19 As well as these STs, according to the strain information in the Pubmlst database (http://pubmlst.org/abaumannii), ST53 and ST4 correspond to multiresistant A. baumannii strains from Italy, and ST33 corresponds to a blaOXA-40-producing A. baumannii from Portugal. Notably, the whole-genome sequencing of multidrug-resistant A. baumannii ACICU belonging to European clone II indicated the presence of an antibiotic resistance island AbaR2 and as many as 36 putative efflux pumps. We propose that our results just reflect one mode of evolution from CSAB to CRAB through horizontal transfer of blaOXA-23.

It has been shown that ISAba1 upstream of blaAmpC contributes to the overexpression of cephalosporinase, which confers resistance to ceftazidime.26 It is interesting that not all of the ceftazidime-resistant isolates harboured this genetic structure in this study. The results imply there may be extended-spectrum β-lactamases present, e.g. PER-1 or SHV, or another IS element as a promoter of expression, e.g. ISaba125 in A. baumannii ACICU.34 The isolates of different STs of CC22 even showed a variable presence of ISaba1 upstream of blaAmpC, indicating the diversity during the evolution of these STs.

In conclusion, we have identified the prevalence of CRAB CC22 with serious multidrug resistance in China. The global occurrence of ST22 and probable relatedness between ST22 and European clone II updates our understanding about the cross-continent distribution of A. baumannii, and more attention should be paid to tracking its further dissemination. However, in order to elucidate the evolutionary origins and globally epidemic situation of CRAB, more studies still need to be carried out to expand the STs in the MLST database of A. baumannii.

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Transparency declarations
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Supplementary data
Table S1 and allelic sequences are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References