Transition of $\text{bla}_{\text{OXA-58-like}}$ to $\text{bla}_{\text{OXA-23-like}}$ in Acinetobacter baumannii Clinical Isolates in Southern China: An 8-Year Study

Weiyuan Wu¹*, Yi He², Jian Lu², Yuemei Lu¹, Jinsong Wu¹, Yingxia Liu²

¹ Department of Laboratory Medicine, Shenzhen People’s Hospital, Second Clinical Medical College of Jinan University, Key Laboratory of Pathogenic Microorganism and Bacterial Resistance Surveillance in Shenzhen, Shenzhen, Guangdong, China, ² Department of Infectious Disease, Third People’s Hospital of Shenzhen, Shenzhen, Guangdong, China

* weiywu@163.com

Abstract

Background
The prevalence of carbapenem-resistant Acinetobacter baumannii in hospitals has been increasing worldwide. This study aims to investigate the carbapenemase genes and the clonal relatedness among A. baumannii clinical isolates in a Chinese hospital.

Methods
Carbapenemase genes and the upstream locations of insertion sequences were detected by polymerase chain reaction (PCR), and the clonal relatedness of isolates was determined by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing.

Results
A total of 231 nonduplicate carbapenemase gene-harboring A. baumannii clinical isolates recovered from Shenzhen People’s Hospital, were investigated between 2002 and 2009. $\text{bla}_{\text{OXA-23-like}}$, $\text{bla}_{\text{OXA-58-like}}$, $\text{bla}_{\text{OXA-40-like}}$, and ISAb1-$\text{bla}_{\text{OXA-51-like}}$ were identified in 119, 107, 1, and 4 isolates, respectively. IS1008-ΔISAb3, ISAb3, and ISAb1 were detected upstream of the $\text{bla}_{\text{OXA-58-like}}$ gene in 69, 35, and 3 isolates, respectively. All $\text{bla}_{\text{OXA-23-like}}$ genes but one had an upstream insertion of ISAb1.

$\text{bla}_{\text{OXA-58-like}}$ was the most common carbapenemase gene in A. baumannii before 2008, thereafter $\text{bla}_{\text{OXA-23-like}}$ became rapidly prevalent and replaced $\text{bla}_{\text{OXA-58-like}}$ in 2009. The majority of $\text{bla}_{\text{OXA-58-like}}$-carrying isolates showed lower level of resistance to imipenem and meropenem (minimum inhibitory concentrations (MICs), 1 μg/ml to 16 μg/ml), compared with the majority of $\text{bla}_{\text{OXA-23-like}}$-carrying isolates (MICs, 16 μg/ml to 64 μg/ml for both imipenem and meropenem). All 231 $\text{bla}_{\text{OXA}}$ carbapenemase gene-harboring isolates belonged to 14 PFGE types (A–N), and three dominant clones A, J, and H accounted for 43.3%, 42.0%, and 8.2% of the tested isolates, respectively. Clone A (sequence type ST92/ST208) with $\text{bla}_{\text{OXA-58-like}}$ was the most prevalent before 2008. Clone H (ST229) with $\text{bla}_{\text{OXA-23-like}}$ became striking between 2007...
and 2008. Clone J (ST381) with \(\text{bla}_{OXA-23}\)-like rapidly spread and replaced clones A and H in 2009.

**Conclusion**

This study is the first to reveal that the distinct \(\text{bla}_{OXA-23}\)-carrying \(A.\ baumannii\) ST381 displaced the previously prevalent \(\text{bla}_{OXA-58}\)-carrying \(A.\ baumannii\) ST92/ST208, resulting in the rapidly increasing resistance to carbapenems in \(A.\ baumannii\) in Shenzhen People’s Hospital in 2009.

**Introduction**

Over the past decade, increasing resistance to carbapenems in \(A.\ baumannii\) has been observed worldwide [1]. This increasing resistance is mainly mediated by production of class D (carbapenem-hydrolyzing oxacillinases [CHDLs]) \(\beta\)-lactamases (OXAs) with carbapenemase activity. Currently, six OXAs with carbapenemase activity gene clusters have been described in \(A.\ baumannii\), including \(\text{bla}_{OXA-23}\)-like, \(\text{bla}_{OXA-40}\)-like, \(\text{bla}_{OXA-51}\)-like, \(\text{bla}_{OXA-58}\)-like, \(\text{bla}_{OXA-143}\)-like, and \(\text{bla}_{OXA-235}\)-like genes [2, 3]. Although the hydrolytic efficiencies of these OXA carbapenemases for carbapenems are relatively low [4], various insertion sequences (ISs) upstream of the \(\text{bla}_{OXA}\) carbapenemase genes, including IS\text{Ab}a1, IS\text{Ab}a2, IS\text{Ab}a3, IS18, IS125, IS1008, and IS\text{Ab}a4, provide promoters for the expression of \(\text{bla}_{OXA}\) carbapenemase genes, except for \(\text{bla}_{OXA-40}\)-like and \(\text{bla}_{OXA-143}\)-like genes, and mediate resistance to carbapenems [5–9].

Clonal spread of carbapenem-resistant \(A.\ baumannii\) has been reported worldwide. Three epidemic lineages of \(A.\ baumannii\), commonly referred to as the pan-European clonal lineages (EU I, EU II, and EU III), account for the majority of \(A.\ baumannii\) infections. Strains that belong to EU II (global clone 2) are widespread throughout the world, including China; many epidemiological studies reported the widespread of OXA-58 producers and OXA-23 producers within this lineage [10–12].

In this study, the transition of \(\text{bla}_{OXA-58}\)-like to \(\text{bla}_{OXA-23}\)-like in \(A.\ baumannii\) clinical isolates from a Chinese hospital between 2002 and 2009 was confirmed. The clonal relatedness of isolates was also investigated by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

**Material and Methods**

**Bacterial isolates and antimicrobial susceptibility testing**

All nonduplicate clinical isolates of \(A.\ baumannii\) were recovered from various wards and clinical samples in Shenzhen People’s Hospital (Shenzhen, Guangdong Province, China), a tertiary-care hospital with 1200 beds, over an 8-year period from 2002 to 2009. The isolates were initially identified using the Vitek 2 system (bioMerieux) and assigned to the \(A.\ baumannii\) complex. Identification of \(A.\ baumannii\) was confirmed by the presence of \(\text{bla}_{OXA-51}\)-like intrinsic to this species by using PCR [13–15]. Agar dilution was performed to detect susceptibilities to imipenem and meropenem for all \(A.\ baumannii\) isolates [16]. Isolates with imipenem and/or meropenem minimum inhibitory concentrations (MICs) \(\geq 0.25 \mu g/ml\) were further investigated for the carbapenemase genes. MICs of other 13 antimicrobial agents were also determined by agar dilution for carbapenemase
gene-carrying *A. baumannii* isolates. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the controls.

**Detection of carbapenemase genes and ISs upstream of CHDL genes**

PCR assays for genes coding for known carbapenemases (i.e., *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>SPM</sub>, *bla*<sub>SIM</sub>, *bla*<sub>GIM</sub>, *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-40-like</sub>, *bla*<sub>OXA-58-like</sub>, *bla*<sub>OXA-143-like</sub>, and *bla*<sub>KPC</sub>) were performed as previously described [2, 17–19]. PCR with primers within the ISs (i.e., IS<sub>Aba1</sub>, IS<sub>Aba2</sub>, IS<sub>Aba3</sub>, IS<sub>18</sub>, IS<sub>125</sub>, and IS<sub>1008</sub>) and reverse primers within the CHDL genes [5–9] mapped the upstream locations of ISs.

**PFGE**

PFGE determined the clonal relationships of the carbapenemase gene-carrying *A. baumannii* isolates. PFGE of *Apa*I (New England)-digested genomic DNA was conducted using the GenePath system (Bio-Rad) as previously described [20, 21]. DNA macrorestriction patterns were interpreted according to the criteria described by Tenover et al [22] and cluster analysis was performed using Fingerprint II software (Bio-Rad). Dendrograms for similarity were constructed using the unweighted-pair group method with arithmetic averages. The Dice correlation coefficient was used to analyze any similarities between banding patterns. In brief, isolates that showed zero to three DNA fragment differences and a similarity of ≥ 85% following dendrogram analysis were considered to represent the same PFGE type.

**MLST**

MLST was conducted as previously described [11, 23] for the representative isolates from the prevalent main clones typed by PFGE. In brief, internal fragments of seven housekeeping genes, i.e., *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*, were PCR amplified, purified, and then sequenced with an ABI prism sequencer 3730 (Applied Biosystems). A new primer pair was redesigned (*recA*-F2, 5′-GCAGTTGAAGCCGTATCT-3′ and *recA*-R2, 5′-TTGACCGATACGACGAA-3′) for both amplification and sequencing to obtain the specific PCR products and satisfactory sequencing results. The internal fragments for analysis were still identical to a previous scheme [23]. The sequence of each allele was compared by Basic Local Alignment Search Tool with existing sequences in Pubmlst database and sequence types (STs) were designated according to the allelic profiles (http://pubmlst.org/abaumannii/).

**Results**

**Distribution of carbapenemase genes**

During the study period, 393 nonduplicate clinical isolates of *A. baumannii*, with imipenem and/or meropenem MICs ≥ 0.25 μg/ml, were recovered from 367 colonized or infected inpatients in Shenzhen People’s Hospital. A total of 231 isolates of *bla*<sub>OXA</sub> carbapenemase gene-har­­­oring *A. baumannii* were detected among of them. *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-40-like</sub>, *bla*<sub>OXA-58-like</sub>, *bla*<sub>OXA-143-like</sub>, and IS<sub>Aba1</sub>–*bla*<sub>OXA-51-like</sub> were identified in 119, 107, 1, and 4 single isolates, respectively. *bla*<sub>OXA-58-like</sub> had been the most common carbapenemase gene in *A. baumannii* prior to 2008; thereafter, *bla*<sub>OXA-23-like</sub> remarkably increased and became rapidly prevalent in *A. baumannii* in 2009 (Table 1). IS1008–ΔIS<sub>Aba3</sub>, IS<sub>Aba3</sub>, and IS<sub>Aba1</sub> were found upstream of the *bla*<sub>OXA-58-like</sub> gene in 69, 35, and 3 isolates, respectively. *bla*<sub>OXA-23-like</sub> genes but one had an upstream insertion of IS<sub>Aba1</sub>.
Antibiotic resistance profiles

Table 2 shows the MIC distributions for both imipenem and meropenem and bla_OXA carbapenemase gene-harboring *A. baumannii*. The majority of bla_OXA-58-like-carrying isolates showed lower level of resistance to imipenem (MICs, 1 μg/ml to 16 μg/ml) and meropenem (MICs, 1 μg/ml to 8 μg/ml), compared with bla_OXA-23-like-carrying isolates (MICs, 16 μg/ml to 64 μg/ml for both imipenem and meropenem). Notably, 26 (24.3%) and 27 (25.2%) isolates with bla_OXA-58-like were classified as “susceptible” (MICs, 0.5 μg/ml to 2 μg/ml) and “intermediate” to imipenem, respectively, using the current Clinical Laboratory Standard Institute (CLSI) breakpoint for susceptibility of ≤2 μg/ml and resistance of ≥8 μg/ml. Furthermore, higher susceptible rate of 35.5% (38/107) and intermediate rate of 42.1% (45/107) were observed to meropenem against bla_OXA-58-like-carrying *A. baumannii* isolates. Only 54 (50.5%) and 24 (22.4%) of 107 bla_OXA-58-like-carrying isolates were classified as resistant to imipenem and meropenem, respectively. By contrast, 119 bla_OXA-23-like-carrying isolates were classified as resistant to both imipenem and meropenem. One bla_OXA-40-like-carrying isolate and four ISAba1-bla_OXA-51-like-carrying isolates were classified as resistant to imipenem and meropenem.

### Table 1. *A. baumannii* (Ab) isolates with imipenem and/or meropenem MICs ≥ 0.25 μg/ml from 2002 to 2009.

| Organism (no. of isolates tested) | No. of isolates | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 |
|----------------------------------|----------------|------|------|------|------|------|------|------|------|
| **bla_OXA-58-like-carrying Ab**  | 107 (90)a      | 4    | 32   | 26   | 8    | 8    | 13   | 12   | 7    | 5    |
| **bla_OXA-23-like-carrying Ab**  | 119 (116)      | 1    | 3    | 6    | 8    | 8    | 1    | 1    | 3    |
| **bla_OXA-40-like-carrying Ab**  | 1 (1)          |      |      |      |      |      |      |      | 1    |
| **ISAba1-bla_OXA-51-like-carrying Ab** | 4 (4)        |      |      |      |      |      |      |      | 1    | 3    |
| **Noncarbapenemase gene-carrying Ab** | 162 (156) | 36   | 20   | 11   | 32   | 16   | 16   | 18   | 13   |
| **Total**                        | 393 (367)      | 40   | 52   | 37   | 41   | 32   | 35   | 34   | 122  |

a Parentheses refer to the number of patients

doi:10.1371/journal.pone.0137174.t001

### Table 2. MIC distributions of imipenem and meropenem against *A. baumannii* (Ab) isolates with or without carbapenemase gene.

| Organism (no. of isolates tested) | 0.125 | 0.25 | 0.5 | 1   | 2   | 4   | 8   | 16  | 32  | 64  | 128 |
|----------------------------------|-------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **bla_OXA-58-like-carrying Ab**  |       |      |     |     |     |     |     |     |     |     |     |
| Imipenem                         | 2     | 11   | 13  | 27  | 38  | 15  | 1   |
| Meropenem                         | 2     | 18   | 18  | 45  | 21  | 2   | 1   |
| **bla_OXA-23-like-carrying Ab**  |       |      |     |     |     |     |     |     |     |     |     |
| Imipenem                         | 31    | 36   | 52  |
| Meropenem                         |       |      |     |     |     |     |     |     |     |     |     |
| **bla_OXA-40-like-carrying Ab**  |       |      |     |     |     |     |     |     |     |     |     |
| Imipenem                         | 8     | 24   | 55  | 29  | 3   |
| Meropenem                         | 1     |
| **ISAba1-bla_OXA-51-like-carrying Ab** | 6 (4) |      |     |     |     |     |     |     |     |     |     |
| Imipenem                         | 1     | 2    |
| Meropenem                         | 1     |
| **Noncarbapenemase gene-carrying Ab** | 162a |      |     |     |     |     |     |     |     |     |     |
| Imipenem                         | 3     | 31   | 18  | 85  | 23  | 2   |
| Meropenem                         | 14    | 26   | 72  | 46  | 4   |

a *A. baumannii* isolates with imipenem and/or meropenem MICs ≥ 0.25 μg/ml

doi:10.1371/journal.pone.0137174.t002
Table 3. Susceptibilities of 15 antimicrobial agents against blaOXA-58-like-carrying and blaOXA-23-like-carrying A. baumannii (Ab).

| Antimicrobial agents | blaOXA-58-like-carrying Ab (n = 107) | blaOXA-23-like-carrying Ab (n = 119) |
|----------------------|-------------------------------------|-------------------------------------|
|                       | R% | I% | S% | MIC50 (µg/ml) | MIC50 (µg/ml) | MIC Range (µg/ml) | R% | I% | S% | MIC50 (µg/ml) | MIC50 (µg/ml) | MIC Range (µg/ml) |
| Imipenem              | 50.5 | 25.2 | 24.3 | 8 | 16 | 0.5–32 | 100 | 0 | 0 | 32 | 64 | 16–64 |
| Meropenem             | 22.4 | 42.1 | 35.5 | 4 | 8 | 0.5–32 | 100 | 0 | 0 | 32 | 64 | 8–128 |
| Cefoperazone-sulbactam | 19.6 | 14 | 66.4 | 16 | 64 | 4–128 | 95.8 | 2.5 | 1.7 | 128 | 128 | 16–256 |
| Ampicillin-sulbactam   | 36.4 | 58.9 | 4.7 | 16 | 64 | 4–128 | 100 | 0 | 0 | 128 | 128 | 32–256 |
| Cefepime              | 49.5 | 46.7 | 3.7 | 16 | 64 | 2–256 | 99.2 | 0.8 | 0 | 64 | 128 | 16–256 |
| Piperacillin-tazobactam | 97.2 | 1.9 | 0.9 | 256 | >256 | 1–256 | 100 | 0 | 0 | >256 | >256 | 128–256 |
| Ceftazidime           | 99.1 | 0 | 0.9 | >256 | >256 | 4–256 | 99.2 | 0.8 | 0 | >256 | >256 | 16–256 |
| Ceftriazone           | 94.4 | 5.6 | 0 | >256 | >256 | 16–256 | 98.3 | 1.7 | 0 | >256 | >256 | 16–256 |
| Amikacin             | 75.7 | 15.9 | 8.4 | 256 | >256 | 1–256 | 82.4 | 12.6 | 5 | >256 | >256 | 1–256 |
| Ciprofloxacin        | 97.2 | 0 | 2.8 | >32 | >32 | 0.25–32 | 100 | 0 | 0 | >32 | >32 | 16–32 |
| Levofloxacin         | 77.6 | 18.7 | 3.7 | 16 | 32 | 0.125–32 | 89.1 | 10.9 | 0 | 16 | 16 | 4–32 |
| Trimethoprim-sulfamethoxazole | 96.3 | 0.3 | >16 | >16 | 0.125–16 | 99.2 | 0 | 0.8 | >16 | >16 | 0.5–16 |
| Polymixin B          | 0 | 0 | 100 | 1 | 1 | 0.5–2 | 0 | 0 | 100 | 1 | 1 | 0.5–1 |
| Minocycline          | 12.1 | 61.7 | 26.2 | 8 | 16 | 0.125–16 | 0.8 | 1.7 | 97.5 | 4 | 4 | 0.5–16 |
| Tigecycline          | 10.3 | 72.9 | 16.8 | 4 | 8 | 2–16 | 4.2 | 79 | 16.8 | 4 | 4 | 2–8 |

a CLSI (2007) breakpoint for cepofoprazone was used for cepofoprazone-sulbactam in this study.

b U.S. FDA criteria for tigecycline were used in this study (susceptibility is defined as ≤2 µg/ml; resistance as ≥8 µg/ml).

R, resistant; I, intermediate; S, susceptible

doi:10.1371/journal.pone.0137174.0003

Carrying isolates were classified as intermediate or resistant to imipenem and meropenem. Majority of A. baumannii isolates without carbapenemase gene were classified as susceptible to imipenem and meropenem, except for the two (1.2%) and four (2.5%) isolates classified as intermediate to imipenem and meropenem, respectively.

blαOXA-58-like-carrying isolates showed moderate susceptibility to a few noncarbapenems (Table 3). More than half of blαOXA-58-like-carrying isolates were still susceptible or intermediate to cefoprazone-sulbactam, ampicillin-sulbactam, and cepofepime, compared with less than 5% of blαOXA-23-like-carrying isolates. Both blαOXA-58-like-carrying isolates and blαOXA-23-like-carrying isolates were highly resistant to piperacillin-tazobactam, ceftazidime, ceftriazone, amikacin, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole (resistance rates, 75.7% to 100%). However, these isolates all exhibited low resistance to polymixin B, minocycline, and tigecycline (resistance rate of less than 15%). The MIC distributions of imipenem and meropenem for IS1008-ΔISAb3-blαOXA-58-like-carrying A. baumannii were similar to those for ISAb3-blαOXA-58-like-carrying A. baumannii (Table 4).

PFGE and MLST

All blαOXA carbapenemase gene-harboring isolates belonged to 14 PFGE types (A–N). Three dominant PFGE-defined clones A, J, and H comprised 100 (43.3%), 97 (42.0%), and 19 (8.2%) isolates, respectively. Clone A with blαOXA-58-like had been the most prevalent prior to 2008. Clone H with blαOXA-23-like became notable between 2007 and 2008. Clone J with blαOXA-23-like rapidly increased and became the dominant clone in place of clones A and H in 2009 (Table 5). Ten representative isolates of the three dominant clones A, J, and H, which were obtained from
ten inpatients, belonged to three different sequence types ST92/ST208, ST381, and ST229, respectively. ST229 was different from ST92/ST208 and ST381 by six alleles. Only two allelic (gyrB and gpi) differences were observed between ST381 and ST92/ST208 (Fig 1), both of which belong to global clone 2.

ST381 (clone J) showed apparently different resistance profiles compared with ST92/ST208 (clone A) (Table 6). ST381 isolates were uniformly resistant to all β-lactam drugs tested. By contrast, ST92/ST208 isolates showed variable resistance to imipenem, meropenem, cefopazone-sulbactam, ampicillin-sulbactam, and cefepime.

**Discussion**

bla\textsubscript{OXA-23-like} carbapenemase genes are disseminated worldwide [1]. In China, bla\textsubscript{OXA-23-like} is the most common carbapenemase gene in A. baumannii, with more than 90% of imipenem-

---

**Table 4.** MIC distributions of imipenem and meropenem against A. baumannii (Ab) isolates with various ISs upstream of the bla\textsubscript{OXA-58-like}.

| Ab with IS upstream of the bla\textsubscript{OXA-58-like} (no. of isolates tested) | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|
| IS\textsubscript{1008-ΔIS\textsubscript{Aba3-bla\textsubscript{OXA-58-like}}} (69) | | | | | | | |
| imipenem | 2 | 5 | 20 | 34 | 8 | | |
| meropenem | 5 | 11 | 39 | 12 | 1 | 1 | |
| IS\textsubscript{Aba3-bla\textsubscript{OXA-58-like}} (35) | | | | | | | |
| imipenem | 2 | 9 | 8 | 7 | 3 | 5 | 1 |
| meropenem | 2 | 13 | 7 | 6 | 6 | 1 | |
| IS\textsubscript{Aba1-bla\textsubscript{OXA-58-like}} (3) | | | | | | | |
| imipenem | 1 | 2 | | | | | |
| meropenem | 3 | |

doi:10.1371/journal.pone.0137174.t004

---

**Table 5.** PFGE types of carbapenemase gene-carrying A. baumannii (Ab) from 2002 to 2009.

| Year | IS\textsubscript{1008-ΔIS\textsubscript{Aba3-bla\textsubscript{OXA-58-like}}} carrying Ab (69) | IS\textsubscript{Aba3-bla\textsubscript{OXA-58-like}} carrying Ab (35) | PFGE type (No.) | bla\textsubscript{OXA-23-like} carrying Ab (119) | bla\textsubscript{OXA-51-like} carrying Ab (4) | bla\textsubscript{OXA-40-like} carrying Ab (1) |
|---|---|---|---|---|---|---|
| 2002 (n = 4) | A (3), B (1) | | | | | |
| 2003 (n = 32) | A (17) | A (13), E (2) | | | | |
| 2004 (n = 26) | A (17) | A (8), F (1) | | | | |
| 2005 (n = 9) | A (6) | A (1), G (1) | I (1) | | | |
| 2006 (n = 16) | A (13) | | H (3) | | | |
| 2007 (n = 19) | A (10) | A (1), C (1) | H (6) | L (1) | | |
| 2008 (n = 16) | A (4) | A (2), D (1) | H (6), I (1), J (1) | I (1) | | |
| 2009 (n = 109) | A (2) | A (3) | H (4), J (96), K (1) | M (2), N (1) | | |

doi:10.1371/journal.pone.0137174.t005
nonsusceptible *A. baumannii*-harbored bla<sub>OXA-23</sub> [11, 24, 25]. In the present study, 119 (30.1%) and 107 (27.2%) of 393 *A. baumannii* isolates with imipenem and/or meropenem MICs ≥ 0.25 μg/ml carried bla<sub>OXA-23-like</sub> and bla<sub>OXA-58-like</sub>, respectively. Surprisingly, bla<sub>OXA-58-like</sub> had been the most common carbapenemase gene in *A. baumannii* in Shenzhen People’s Hospital until 2008. bla<sub>OXA-23-like</sub> occurred in a sporadic clone I for the first time in the hospital in 2005 and then remarkably increased and became rapidly prevalent in *A. baumannii* clone J.

![PFGE dendrogram of 10 representative isolates from the three dominant clones.](doi:10.1371/journal.pone.0137174.g001)

**Table 6. Antibiotic resistance profiles of the three main carbapenemase gene-harboring *A. baumannii* clones (MIC μg/ml).**

| Antimicrobial Agents | Clone A/ST92/ST208 (n = 100) | Clone J/ST381 (n = 97) | Clone H/ST229 (n = 19) |
|----------------------|-------------------------------|------------------------|------------------------|
|                      | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC Range | R% | S% | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC Range | R% | S% | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC Range | R% | S% | MIC<sub>50</sub> | MIC<sub>90</sub> | MIC Range |
| IPM                  | 64 | 64 | 1–32 | 64 | 64 | 16–64 | 64 | 64 | 16–64 | 64 | 64 | 16–64 |
| MEM                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| CSL<sup>a</sup>      | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| SAM                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| FEP                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| TZP                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| CAZ                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| CRO                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| AMK                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| CIP                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| LEV                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| SXT                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| POL                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| MNO                  | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |
| TGC<sup>b</sup>      | 64 | 64 | 1–32 | 128 | 128 | 64–256 | 128 | 128 | 64–256 | 128 | 128 | 64–256 |

IPM, imipenem; MEM, meropenem; CSL, cefoperazone-sulbactam; SAM, ampicillin-sulbactam; FEP, cefepime; TZP, piperacillin-tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; AMK, amikacin; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; POL, polymixin B; MNO, minocycline; TGC, tigecycline.

R, resistant; S, susceptible

<sup>a</sup> CLSI (2007) breakpoint for cefoperazone was used for cefoperazone-sulbactam in this study.

<sup>b</sup> U.S. FDA criteria for tigecycline were used in this study (susceptibility is defined as ≤ 2 μg/ml; resistance as ≥ 8 μg/ml).

doi:10.1371/journal.pone.0137174.t006
in 2009. Notably, the similar replacement of \(\text{bla}_{\text{OXA}}\) carbapenemase genes in \textit{A. baumannii} was reported in Italy during the same period [10, 26]. We also found that the majority of \(\text{bla}_{\text{OXA}}\)-58-like-carrying isolates showed lower level of resistance to carbapenems compared with \(\text{bla}_{\text{OXA}}\)-23-like-carrying isolates. Only 54 (50.5%) and 24 (22.4%) of 107 \(\text{bla}_{\text{OXA}}\)-58-like-carrying isolates were classified as resistant to imipenem and meropenem, respectively, using the current CLSI breakpoint. By contrast, all 119 \(\text{bla}_{\text{OXA}}\)-23-like-carrying isolates were classified as resistant to both imipenem and meropenem. Less \(\text{bla}_{\text{OXA}}\)-58-like-carrying isolates would be classified as resistant to imipenem (16/107) and meropenem (3/107) using the previous CLSI breakpoint for susceptibility of \(\leq 4 \, \mu g/ml\) and resistance of \(\geq 16 \, \mu g/ml\) [27]. Interestingly, \(\text{bla}_{\text{OXA}}\)-58-like-carrying isolates showed moderate susceptibility to cefoperazone-sulbactam, ampicillin-sulbactam, and cefepime compared with \(\text{bla}_{\text{OXA}}\)-23-like-carrying isolates, which were highly resistant to these drugs in the present study. Coelho et al. [28] examined 28 isolates of \(\text{bla}_{\text{OXA}}\)-58-like-carrying \textit{A. baumannii} collected worldwide. They found that imipenem and meropenem MICs of 1–4 \(\mu g/ml\) were detected in 17 and 22 isolates, respectively. The carbapenem MICs varied from 32 \(\mu g/ml\) to 1–4 \(\mu g/ml\) for the isolates from different countries. Based on these findings, we speculate that some \(\text{bla}_{\text{OXA}}\)-58-like-carrying \textit{A. baumannii} isolates may spread undetected in previous studies from China because of the relatively low imipenem and/or meropenem MICs for these organisms.

The flanking IS elements IS\text{Aba}1, IS\text{Aba}2, IS\text{Aba}3, IS\text{Aba}825, IS18, and IS1008 regulate \(\text{bla}_{\text{OXA}}\)-58-like gene expression. Meanwhile, the latter four all provide hybrid promoters, as described in the recent studies [5, 6, 8, 29]. IS1008-IS\text{Aba}3 was the most common IS upstream of the \(\text{bla}_{\text{OXA}}\)-58-like gene in \textit{A. baumannii} clinical isolates in this study, followed by IS\text{Aba}2 and IS\text{Aba}1. Chen et al. reported that a single plasmid-borne IS1008-IS\text{Aba}3-\(\text{bla}_{\text{OXA}}\)-58 is enough to confer a high level of resistance to carbapenem for \textit{A. baumannii}. The insertion of IS1008 provided a hybrid promoter and increased the transcription level of the \(\text{bla}_{\text{OXA}}\)-58 gene [8]. However, the present study found that IS1008-IS\text{Aba}3-\(\text{bla}_{\text{OXA}}\)-58-like-harboring \textit{A. baumannii} isolates showed variable susceptibility to carbapenems (MICs 1 \(\mu g/ml\) to 32 \(\mu g/ml\)). Meanwhile, the similar carbapenem MIC distributions were also detected in IS\text{Aba}3-\(\text{bla}_{\text{OXA}}\)-58-like-harboring \textit{A. baumannii} isolates (MICs 0.5 \(\mu g/ml\) to 32 \(\mu g/ml\)). The reasons for the variation in the resistance levels remain unknown. Several previous studies demonstrated that the overexpression of the AdeABC efflux pump and expression of OXA-23 or OXA-58 lead to higher levels of carbapenem resistance [26, 30–32]. In addition, Bertini et al. [33] described that the multiple copies of \(\text{bla}_{\text{OXA}}\)-58 increase the level of resistance to carbapenems. However, the study of D’Arezzo showed the opposite conclusion; they reported that the resistance to meropenem or imipenem is not associated with \(\text{bla}_{\text{OXA}}\)-58-like gene copy number per plasmid or to loss of integrity of the CarO porin [26]. Taken together, we speculate that the variable IS upstream of the \(\text{bla}_{\text{OXA}}\)-58-like gene, high copy number of \(\text{bla}_{\text{OXA}}\)-58-like overexpression of efflux system, and other cofactors may confer a high level of resistance to carbapenem in \textit{A. baumannii}. In addition, neither IS\text{Aba}1 nor IS\text{Aba}4 was detected upstream of the \(\text{bla}_{\text{OXA}}\)-23-like gene in one isolate in the current study, though several attempts were conducted. This result may be due to another unknown resistance mechanism, which confers resistance to carbapenems in this isolate.

\textit{A. baumannii} clonal complex 92, corresponding to the global clone 2, has been found worldwide [34], which comprises more than 100 STs, including ST75, ST92, ST92/ST208, and ST381. To the best of our knowledge, ST75, ST92, and ST92/ST208 were the most common STs in China, and ST381 was first identified as sporadic clone in two hospitals in Sichuan, Southwest China in 2011. All of these STs harbored \(\text{bla}_{\text{OXA}}\)-23 gene [11, 12]. Notably, the present study demonstrated the prevalence of ST92/ST208 with \(\text{bla}_{\text{OXA}}\)-58-like in Shenzhen People’s Hospital prior to 2008. Surprisingly, ST381 with \(\text{bla}_{\text{OXA}}\)-23-like first emerged in this hospital on December 30, 2008; thereafter, it rapidly spread and replaced the ST92/ST208 and ST229 with
*bl*OXA-23-like* in 2009. ST229 occurred for the first time in this hospital in 2006 and became one of the main clones in 2007 and 2008, which is genetically completely unrelated to ST92/ST208 and ST381. The reasons for the prevalence of clone J (ST381) are still unknown. Clone J was first isolated from the sputum of a 74-year-old male diabetes inpatient, who had been artificially ventilated for seven days in intensive care unit (ICU) for severe community-acquired pneumonia. The reinfection of *A. baumannii* was confirmed by infectious-disease physicians with subsequent several positive sputum cultures, clinical symptoms and signs, and effective responses to antibiotic therapy against *A. baumannii* with cefoperazone-sulbactam. This patient impossibly introduced the ST381 strain with *bl*OXA-23-like in the hospital, because both of his two sputum cultures obtained on the first and fifth days of his hospitalization in the ICU showed negative results. This strain possibly survived in the ICU environment prior to this infection. By investigating the usage of carbapenems in the inpatients of Shenzhen People’s Hospital from 2004 to 2009, 1.8 and 2.2-fold increase of defined daily doses (DDDs) of imipenem (1521.25 to 2777.5) and meropenem (1464.75 to 3232.5) were observed in 2009, respectively. In particular, the DDDs of meropenem had been maintained higher than those of imipenem since 2006 (S1 Fig). Neither a change in the hospital policy nor the introduction of a new antibiotic was observed during this period. We hypothesized that the increasing selective pressure in this hospital environment screened the clone J with *bl*OXA-23-like, which subsequently caused the huge outbreak in 2009. Minandri et al. [10] investigated the transition of *bl*OXA-58 to *bl*OXA-23 gene carriage from 2005 to 2009 among *A. baumannii* isolates responsible for ICU outbreaks in the main hospitals of central Italy. They found that all isolates from the transition period demonstrate extensive genetic similarity, all belonging to ST2 determined by the scheme of Daincourt et al [35]. Interestingly, the present study also indicates the occurrence of clone replacement between genetically similar ST381 and ST92/ST208. We speculate that the higher carbapenemase activity of OXA-23-like compared with OXA-58-like, may provide *bl*OXA-23-like-carrying ST 381 with a selective advantage over *bl*OXA-58-like-carrying ST92/ST208 by increasing the resistance to both imipenem and meropenem. However, the dominant role of ST 381 remains unknown among the *A. baumannii* population in the short period other than ST229, although the latter occurred earlier. Further study is needed to elucidate this question.

**Conclusion**

We first reported the distinct *bl*OXA-23-like-carrying *A. baumannii* ST381 with high level of resistance to carbapenems, which rapidly spread and replaced the previously prevalent *bl*OXA-58-like-carrying ST92/ST208 with variable susceptibility to carbapenems, resulting in the increased resistance to carbapenems in *A. baumannii* in a Chinese hospital in 2009.

**Supporting Information**

S1 Fig. DDDs of imipenem and meropenem from 2004 to 2009. (TIF)

**Author Contributions**

Conceived and designed the experiments: WYW JL YXL. Performed the experiments: WYW YH. Analyzed the data: WYW YH YML JSW JL. Contributed reagents/materials/analysis tools: JL WYW YML JSW YXL. Wrote the paper: WYW YH.
References

1. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of Acinetobacter baumannii. Emerg Infect Dis. 2010; 16:35–40. doi: 10.3201/eid1601.090852 PMID: 20031040

2. Higgins PG, Lehmann M, Seifert H. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2010; 35:305–314.

3. Higgins PG, Pérez-Llarena FJ, Zander E, Fernández A, Bou G, Seifert H. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in Acinetobacter baumannii. Antimicrob Agents Chemother. 2013; 57:2121–2126. doi: 10.1128/AAC.02413-12 PMID: 23439638

4. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-Lactamases. Antimicrob Agents Chemother. 2010; 54:24–36. doi: 10.1128/AAC.01512-08 PMID: 19721065

5. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett. 2006; 258:72–77. PMID: 16830258

6. Poirel L, Nordmann P. Genetic structure at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-58 in Acinetobacter baumannii. Antimicrob Agents Chemother. 2006; 50:1442–1448. PMID: 16569863

7. Evans BA, Hamouda A, Towner KJ, Amyes SGB. Novel genetic context of multiple blaOXA-58 genes in Acinetobacter genospecies 3. J Antimicrob Chemother. 2010; 65:1586–1588. doi: 10.1093/jac/dkq180 PMID: 20542900

8. Chen TL, Wu RC, Shaio MF, Fung CP, Cho WL. Acquisition of a plasmid-borne blaOXA-58 gene with an upstream IS1008 insertion conferring a high level of carbapenem resistance to Acinetobacter baumannii. Antimicrob Agents Chemother. 2008; 52:2573–2580. doi: 10.1128/AAC.00939-08 PMID: 18443121

9. Corvec S, Poirel L, Naas T, Drugeon H, Nordmann P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in Acinetobacter baumannii. Antimicrob Agents Chemother. 2007; 51:1530–1533. PMID: 17220422

10. Minandi F, D’Arezzo S, Antunes LC, Pourcel C, Principe L, Petrosillo N, et al. Evidence of diversity among epidemiologically related carbapenemase-producing Acinetobacter baumannii strains belonging to international clonal lineage II. J Clin Microbiol. 2012; 50:590–597. doi: 10.1128/JCM.05555-11 PMID: 22205821

11. Wang X, Qiao F, Yu R, Gao Y, Zong Z. Clonal diversity of Acinetobacter baumannii clinical isolates revealed by a snapshot study. BMC Microbiol. 2013; 13:234. doi: 10.1186/1471-2180-13-234

12. Fu Y, Zhou J, Hou H, Yang Q, Wei Z, Yu Y, et al. Wide dissemination of OXA-23-producing carbapenem-resistant Acinetobacter baumannii clonal complex 22 in multiple cities of China. J Antimicrob Chemother. 2010; 65:644–650. doi: 10.1093/jac/dkq027 PMID: 20154023

13. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like gene intrinsic to this species. J Antimicrob Agents Chemother. 2006; 44:2974–2976. PMID: 16891520

14. Turton JF, Shah J, Ozyongwu C, Pike R. Incidence of Acinetobacter species other than A. baumannii among clinical isolates of Acinetobacter evidence for emerging species. J Clin Microbiol. 2010; 48:1445–1449. doi: 10.1128/JCM.02467-09 PMID: 20181894

15. Wang J, Ruan Z, Peng Y, Fu Y, Jiang Y, Wang H, et al. Species distribution of clinical Acinetobacter isolates revealed by different identification techniques. PLoS One 2014; 9(8):e104882. doi: 10.1371/journal.pone.0104882 PMID: 25120020

16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 24th informational supplement. M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA; 2014.

17. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. J Antimicrob Chemother. 2007; 59:321–322. PMID: 17185300

18. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2006; 27:351–353. PMID: 16564159

19. Robledo IE, Aquino EE, Santé MI, Santana JL, Otero DM, León CF, et al. Detection of KPC in Acinetobacter spp. in Puerto Rico. Antimicrob Agents Chemother. 2010; 54:1354–1357. doi: 10.1128/AAC.00899-09 PMID: 20386118

20. Hunter SB, Vauterin P, Lambert-Fair MA, Duyne MSY, Kubota K, Graves L, et al. Establishment of a Universal Size Standard Strain for Use with the PulseNet Standardized Pulsed-Field Gel
Electrophoresis Protocols: Converting the National Databases to the New Size Standard. J Clin Microbiol. 2005; 43:1045–1050. PMID:15750058

21. Seifert H, Dolzani L, Bressan R, van der Reijden T, van Strijen B, Stefanik D, et al. Standardization and Interlaboratory Reproducibility Assessment of Pulsed-Field Gel Electrophoresis-Generated Fingerprints of Acinetobacter baumannii. J Clin Microbiol. 2005; 43:4328–4335. PMID:16145073

22. Tenover FC, Arbet RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995; 33:2233–2239. PMID:7494007

23. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol. 2005; 43:4382–4390. PMID:16145081

24. Zhou H, Yang Q, Yu YS, Wei ZQ, Li LJ. Clonal spread of imipenem-resistant Acinetobacter baumannii among different cities of China. J Clin Microbiol. 2007; 45:4054–4057. PMID:17942662

25. Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. Molecular epidemiology of clinical isolates of carbapenem-resistant Acinetobacter spp. from Chinese hospitals. Antimicrob Agents Chemother. 2007; 51: 4022–4028 PMID:17846127

26. D’Arezzo S, Principe L, Capone A, Petrosillo N, Petrucca A, Visca P. Changing carbapenemase gene pattern in an epidemic multidrug-resistant Acinetobacter baumannii lineage causing multiple outbreaks in central Italy. J Antimicrob Chemother. 2011; 66:54–61. doi:10.1093/jac/dkq407 PMID: 21088019

27. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 23rd informational supplement. M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

28. Coelho J, Woodford N, Afzal-Shah M, Livermore D. Occurrence of OXA-58-like carbapenemases in Acinetobacter spp. collected over 10 years in three continents. Antimicrob Agents Chemother. 2006; 50: 756–758. PMID:16436738

29. Ravasi P, Limansky AS, Rodriguez RE, Viale AM, Mussi MA. ISAba825, a functional insertion sequence modulating genomic plasticity and blaOXA-58 expression in Acinetobacter baumannii. Antimicrob Agents Chemother. 2011; 55:917–920. doi:10.1128/AAC.00491-10 PMID:21098239

30. Heritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacilllnases to carbapenem resistance in Acinetobacter baumannii. Antimicrob Agents Chemother. 2005; 49: 3198–3202. PMID:16048925

31. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006; 12: 826–836. PMID:16882287

32. Higgins PG, Wisplinghoff H, Stefanik D, Seifert H. Selection of topoisomerase mutations and overexpression of adeB mRNA transcripts during an outbreak of Acinetobacter baumannii. J Antimicrob Chemother. 2004; 54: 821–823. PMID:15355942

33. Bertini A, Poirel L, Bernabei S, Fortini D, Villa L, Nordmann P, et al. Multicopy blaOXA-58 gene as a source of high-level resistance to carbapenems in Acinetobacter baumannii. Antimicrob Agents Chemother. 2007; 51: 2324–2328. PMID:17438042

34. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of Acinetobacter baumannii. Emerg Infect Dis. 2010; 16:35–40. doi: 10.3201/eid1601.090852 PMID: 20031040

35. Nemec A, Krízová L, Maixnerová M, Diancourt L, van der Reijden TJ, Brisse S, et al. Emergence of carbapenem resistance in Acinetobacter baumannii in the Czech Republic is associated with the spread of multidrug resistant strains of European clone II. J Antimicrob Chemother. 2008; 62: 484–489. doi: 10.1093/jac/dkn205 PMID:18477708