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# Clonal Spread of Imipenem-Resistant *Acinetobacter baumannii* among Different Cities of China[▽]

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**A total of 342 imipenem-resistant *Acinetobacter baumannii* isolates (IRABs) were collected from 16 Chinese cities. Six predominant clones had spread widely, and four clones were detected in distant hospitals. The majority of the IRABs contain $bla_{OXA-23}$, with IS*Aba1* upstream of the gene. These results suggested that clonal spread played an important role in the outbreak of IRABs in China.**

*A. baumannii* is recognized as an increasingly important opportunistic gram-negative pathogen that is frequently associated with nosocomial outbreaks worldwide (21). Carbapenems are useful agents that could combat many severe *Acinetobacter* infections. Resistance to carbapenems is now accumulating, largely through clonal spread (13). A few lineages even achieve an epidemic status, reaching multiple hospitals or countries (6). One mechanism of resistance to carbapenems is due to the carbapenem-hydrolyzing β-lactamases of molecular classes B and D (12). Four of the eight clusters of OXA-type carbapenemases were identified in *A. baumannii*, including clusters OXA-23, OXA-24, OXA-51, and OXA-58 (22). IS*Aba1* may have an important role in the expression and transfer of OXA-type carbapenemase genes (17, 20).

In this study, we investigated the prevalence of imipenem-resistant *A. baumannii* isolates (IRABs) in China and characterized the genes of the carbapenemases in IRABs. Three hundred forty-two nonduplicate IRABs were recovered from 16 cities through January 2005 to December 2005 in China (Table 1; Fig. 1). All isolates were identified by the Vitek GNI$^+$ card (BioMérieux, Marcy-l'Etoile, France) as members of the *Acinetobacter calcoaceticus-A. baumannii* complex. Species identification was confirmed by sequence analyses of the 16S-23S rRNA gene spacer region (4).

The MICs of 10 antimicrobial agents were determined by the agar dilution technique by following the standards of Clinical and Laboratory Standards Institute (5); these agents included imipenem (Merck KGaA, Darmstadt, Germany), meropenem (Sumitomo Pharmaceuticals Co., Ltd., Osaka, Japan), piperacillin (Sigma, Deisenhofen, Germany), ceftazidime (Sigma, Deisenhofen, Germany), cefepime (Sino-American Shanghai Squibb Pharmaceuticals Ltd., Shanghai, China), aztreonam (Sigma, Deisenhofen, Germany), amikacin (Sigma, Deisenhofen, Germany), gentamicin (Sigma, Deisenhofen, Germany), ciprofloxacin (Bayer, Leverkusen, Germany), and minocycline (Sigma, Deisenhofen, Germany). The MICs of four other agents, ampicillin-sulbactam, cefoperazone-sulbactam, piperacillin-tazobactam, and polymyxin E, were determined by Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. The results were interpreted according to CLSI 2006 guidelines (5). *Pseudomonas aeruginosa* ATCC 27853 was used as a control. All isolates were found to be resistant to imipenem and meropenem. The rates of resistance to ampicillin-sulbactam, cefoperazone-sulbactam, and minocycline were 68.0%, 54.2%, and 75.9%, respectively. The rate of resistance to polymyxin E was 10.8%, the lowest among the tested agents. The rates of resistance to all other tested antimicrobial agents were more than 90%.

To characterize these isolates genetically, the genomic DNA of all the isolates was digested with ApaI and the resultant DNA fragments were subjected to pulsed-field gel electrophoresis (PFGE) as described previously (18). According to the datum-interpreting criteria described by Tenover et al. (19), these *A. baumannii* isolates belonged to 29 distinct clones (Fig. 2). Among them, six clones were dominant, consisting of 303 strains in total (Table 1).

Crude β-lactamase preparations were extracted by the sonication method (25). pI values were determined by the Phast-System electrophoresis system (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. pI reference proteins were stained with Coomassie brilliant blue R-250. The pattern was analyzed by Curve Expert software (version 1.3) developed by Daniel Hyams (free software). OXA-23-producing *A. baumannii* (a collection of our laboratory) was used as the positive control. Metallo-β-lactamase-producing isolates were screened by the imipenem-EDTA double-disk synergy test (10). IMP-4-producing *A. baumannii* and VIM-2-producing *P. aeruginosa* were used as the positive controls. All tested isolates had one band at pI 6.64 and were negative by the imipenem-EDTA double-disk synergy test.

$bla_{IMP-type}$, $bla_{VIM-type}$, $bla_{SIM-1}$, $bla_{OXA-23-like}$, $bla_{OXA-24-like}$, $bla_{OXA-58-like}$, and $bla_{OXA-51-like}$ were analyzed by PCR using primers as described previously (9, 11, 20). PCR mapping was performed using the IS*Aba1*-OXA-23-like primers or the IS*Aba1*-OXA-51-like primers as reported previously (26). The results showed that all the isolates contained $bla_{OXA-51-like}$ but were negative for the OXA-24-like, OXA-58-like, SIM-1, IMP-type, or VIM-type gene. Three hundred twenty-two isolates

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TABLE 1. The PFGE clone distribution of 342 clinical isolates among 16 cities

| City | Total no. of isolates | No. of isolates of PFGE clone | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | Other |
| Beijing | 5 | 2 | | | | | | 3 |
| Shanghai | 30 | | 17 | 10 | | | | 3 |
| Guangzhou | 17 | | | | | 10 | | 7 |
| Shenyang | 15 | | 10 | | | | | 5 |
| Chongqing | 4 | 1 | | | | | | 3 |
| Hangzhou | 149 | 17 | 5 | 97 | 24 | | | 6 |
| Ningbo | 23 | 21 | | 1 | | | | 1 |
| Zhoushan | 1 | 1 | | | | | | |
| Jianxing | 7 | | | 6 | | | | 1 |
| Huzhou | 8 | | | 6 | | | | 2 |
| Quzhou | 8 | | | 6 | | | | 2 |
| Jinghua | 17 | 1 | | | 7 | | 8 | 1 |
| Taizhou | 13 | | | 11 | | | | 2 |
| Lishui | 21 | | | 20 | | | | 1 |
| Shaoxing | 15 | 15 | | | | | | |
| Wenzhou | 9 | 4 | | 3 | | | | 2 |
| Total | 342 | 62 | 32 | 160 | 31 | 10 | 8 | 39 |

contained the $bla_{\text{OXA-23-like}}$ gene. PCR with the IS*Aba1*-OXA-23-like primers generated a PCR product in 314 IRABs, and sequencing analysis showed that the PCR product contained the OXA-23 gene (the GenBank accession number AJ132105), suggesting that a majority of the clones contain the OXA-23 gene with IS*Aba1* upstream of it. PCR with the IS*Aba1*-OXA-51-like primers generated a PCR product, which contained the OXA-66 gene (the GenBank accession number AY750909) in 13 strains, as revealed by sequencing analysis. There was a 34-bp nucleotide sequence between IS*Aba1* and $bla_{\text{OXA-23}}$ and a 7-bp nucleotide sequence between IS*Aba1* and $bla_{\text{OXA-66}}$.

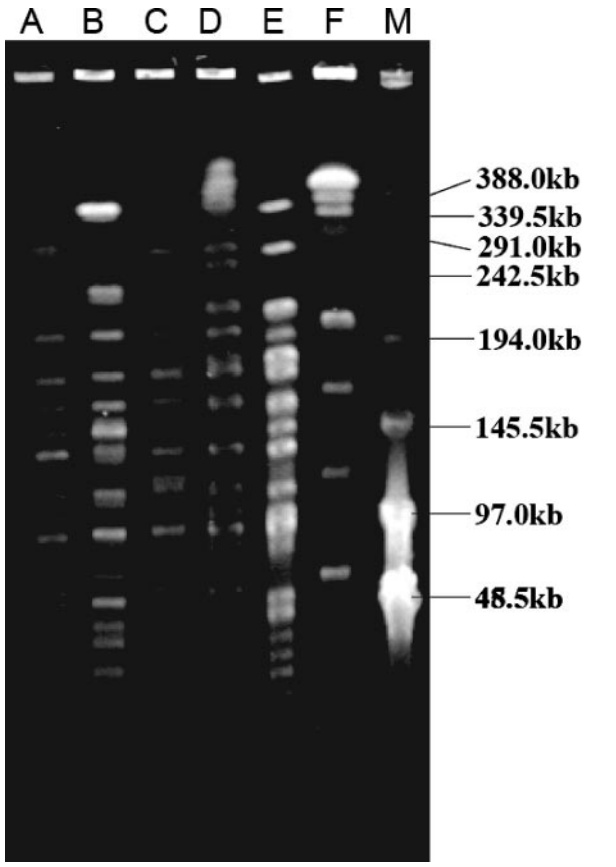


FIG. 2. PFGE analysis of the six major clones of *A. baumannii*. Genomic DNA isolated from the six major *A. baumannii* clones was digested with ApaI before PFGE analysis. Lane M, λ ladder used as molecular size marker; lanes A to F, clones A to F, respectively, of *A. baumannii*.


FIG. 1. The geographic distribution of 16 cities in China. On the left, a map of China shows three autonomous cities (Beijing, Chongqing, and Shanghai) and three provincial cities (Hangzhou [Zhejiang], Guangzhou [Guangdong], and Shenyang [Liaoning]), in which *Acinetobacter baumannii* isolates were collected. On the right, a map shows the 11 cities, including Hangzhou, in Zhejiang Province.

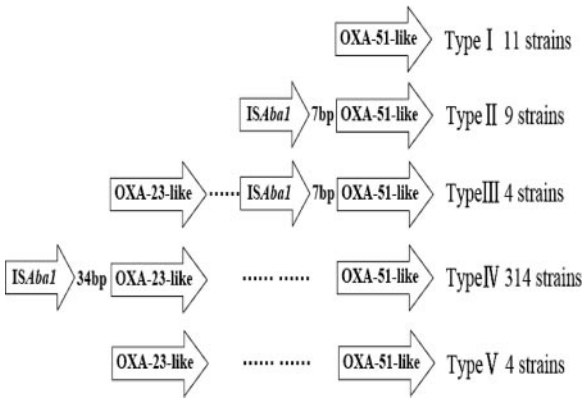


FIG. 3. PCR mapping of $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, and IS*Aba1* in IRABs. According to the PCR mapping results, 342 strains were divided into five types: type I, consisting of 11 strains, contains $bla_{\text{OXA-51-like}}$ without the upstream IS*Aba1*; type II, consisting of 9 strains, contains $bla_{\text{OXA-51-like}}$ with the upstream IS*Aba1*; type III, consisting of 4 strains, contains both $bla_{\text{OXA-51-like}}$ with the upstream IS*Aba1* and $bla_{\text{OXA-23-like}}$ without the upstream IS*Aba1*; type IV, consisting of 314 strains, contains $bla_{\text{OXA-51-like}}$ without IS*Aba1* and $bla_{\text{OXA-23-like}}$ with IS*Aba1*; and type V, consisting of 4 strains, contains $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-23-like}}$ without IS*Aba1* upstream of either gene.

According to the PCR mapping, the 342 strains belonged to five types (Fig. 3).

Attempts to transfer $bla_{\text{OXA-23}}$ by conjugation using the rifampin-resistant *Escherichia coli* 600 as the recipient failed. Repeated attempts to detect the presence of plasmids capable of hybridizing with the $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-66}}$ probes also failed. ApaI-digested PFGE DNAs were transferred to nylon membranes and were hybridized with a v-dCTP (DuPont Corporation)-labeled $bla_{\text{OXA-23}}$ gene probe. The fragments of the ApaI-digested PFGE DNA of pulsotypes A, B, and C showed hybridization bands at about >220 kb in clone A, about >300 kb in clone B, and about 220 kb in clone C, respectively (data not shown). These results are indicative of a chromosomal location for the $bla_{\text{OXA-23}}$ gene.

Our study revealed that IRABs have resistance to most antimicrobial agents. Some studies have indicated that colistin may be useful for treating infections caused by carbapenem-resistant pathogens (14). We confirmed that in vitro, the most active agent against these resistant isolates was polymyxin E.

This is the first report to investigate IRABs in China on such a large scale. We found that clones A, B, C, and D were the dominant isolates and had spread widely among hospitals, cities, and even remote areas. Some isolates with the same genetic basis spread in many hospitals in China, suggesting that the spread of isolates plays an important role in the increase of IRABs. So, it is important to monitor and control the spread of *A. baumannii* having resistance to most β-lactams.

In China, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51-like}}$ were the most popular carbapenemase genotypes. OXA-23-producing IRABs spread widely in the world (1, 2, 7, 9, 16, 23, 24). $bla_{\text{OXA-51-like}}$ genes are the endogenous genes of *A. baumannii*, and their protein products have weak carbapenem-hydrolyzing activities (3, 8). An insertion of IS*Aba1* may provide a promoter for the OXA-type carbapenemase genes to increase their expression (20). There were only 15 isolates (4.39%) that lacked IS*Aba1* upstream of OXA-type carbapenemase coding genes, suggesting that an insertion of IS*Abal* may be a major factor responsible for the carbapenem resistance.

TABLE 2. The distribution of carbapenemase genes in the major PFGE clones of IRABs

| Type | No. of strains of clone | | | | | | | No. of strains |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | Other | |
| I | 1 | | 2 | | | | 8 | 11 |
| II | 3 | | 4 | | | | 2 | 9 |
| III | | | | | | | 4 | 4 |
| IV | 57 | 32 | 152 | 31 | 10 | 8 | 24 | 314 |
| V | 1 | | 2 | | | | 1 | 4 |
| Total (no. of affected cities) | 62 (8) | 32 (3) | 160 (9) | 31 (2) | 10 (1) | 8 (1) | 39 (14) | 342 (16) |

All isolates belonging to clones B, D, E, and F had a PCR mapping of type IV. In contrast, isolates of clones A and C and sporadic isolates had complex PCR mapping results (Table 2). In the process of the dissemination, the strains may have acquired or lost different gene elements under different antimicrobial pressures (15).

**Nucleotide sequence accession numbers.** Sequences for the 34 bp between IS*Aba1* and $bla_{\text{OXA-23}}$ and the 7 bp between IS*Aba1* and $bla_{\text{OXA-66}}$ have been submitted to GenBank under accession numbers DQ923478 (26) and DQ923479, respectively.

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