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# Clonal Spread of Carbapenem Resistant *Acinetobacter baumannii* ST92 in a Chinese Hospital during a 6-Year Period

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**The carbapenem resistance rate of *Acinetobacter baumannii* in our hospital has increased steadily since 2004. The molecular epidemiology of carbapenem resistant *A. baumannii* (CRAB) clinical isolates was characterized by multilocus sequence typing (MLST) and rep-PCR in parallel, with pan-drug susceptible *A. baumannii* (PSAB) used as control. MLST was performed to determine the sequence types (STs), and eBURST algorithm was used to analyze their relatedness. Carbapenem resistance related genes (*oxa-23*, *oxa-24*, *oxa-51*, *oxa-58*, *imp*, *vim*, and *adeB*) were screened using Polymerase Chain Reaction (PCR) method. 23 STs were identified in the 65 included isolates, with ST92 being the predominant clone. PSAB clustered into more singletons than CRAB. The positivity of *oxa-23* and *adeB* correlated with high level carbapenem resistance ($MIC_{IPM}$>32 mg/L, $MIC_{MEM}$ >32 mg/L) of CRAB ST92 isolates in 2009, which was different from the resistance pattern ($MIC_{IPM}$≤4 mg/L, 8 mg/L ≤$MIC_{MEM}$≤16 mg/L) of CRAB ST92 isolates in 2004. These observations suggest that clonal spread of CRAB ST92 isolates longitudinally is the possible reason for carbapenem resistance rate increase and correlate with high level carbapenem resistance in our hospital.**

***Keywords*:** *Acinetobacter baumannii*, carbapenem resistance, Multilocus Sequence Typing (MLST), clonal spread

## Introduction

*Acinetobacter baumannii* is emerging as an important nosocomial pathogen worldwide, especially in intensive care unit (ICU), and can cause serious infections such as ventilator-associated pneumonia, septicemia, urinary tract infection, nosocomial meningitis and wound infection (Peleg *et al*., 2008). It has the ability to acquire resistance to various useful antimicrobial agents, and multidrug-resistant *A. baumannii* increased dramatically. Carbapenems are often the last resort to treat these multidrug-resistant isolates, however, the incidence of carbapenem resistanct *A. baumannii* (CRAB) has risen dramatically and is considered a global sentinel event (Munoz-Price and Weinstein, 2008). CRAB correlates with pan-drug resistance in most circumstances, thus empirical treatment choices are extremely limited.

Understanding the molecular epidemiology is necessary to better control CRAB. Pulsed field gel electrophoresis (PFGE) has been considered the gold standard for *A. baumannii* typing (Seifert *et al*., 2005), however, the interlaboratory exchange of its typing results for global analysis is not feasible. Multilocus Sequence Typing (MLST) of *A. baumannii* developed and put the data into global context (Bartual *et al*., 2005), Previous studies documented that 3 major clonal complexes spreading in Europe, North America, Asia, African, and Australia (van Dessel *et al*., 2004), the successful clones were possibly due to international spread of patients. However, the data from China mainland were still limited, especially those from one institution for longer period of time.

Peking University First Hospital is 1500-bed teaching hospital of Peking University in Beijing, China, and patient visits increased steadily over the past decade. The incidence of CRAB was relatively low before 2004, and increased since 2004, just as we previously reported (Huang *et al*., 2008). The resistance and intermediate rate to carbapenems (imipenem and meropenem) rose to approximately 60% in 2009 (Fig. 1). Understanding the molecular epidemiology of CRAB over longer period may provide new insights into the behavior of this emerging pathogen. In this study, we have undertaken a longitudinally epidemiologic evaluation of CRAB isolated from different wards of our hospital during a 6-year period.

## Materials and Methods

### Bacterial isolates

The carbapenem resistant *A. baumannii* (CRAB) and pan-drug susceptible *A. baumannii* (PSAB) clinical isolates from

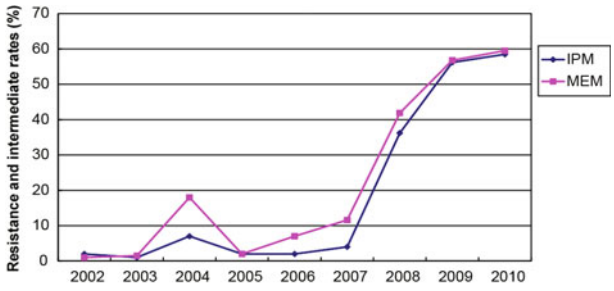


**Fig. 1. Imipenem (IPM) and meropenem (MEM) resistance and intermediate rates of *A. baumannii* during 2002 and 2010 in our hospital.**

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**Table 1. Characteristics, carbapenem susceptibility and molecular typing of CRAB and PSAB isolates**

| Number of isolates | | MLST typing | Rep-PCR typing | Carbapenem MICs (mg/L) | | Carbapenem resistance genes | | Years of isolation | Wards |
|---|---|---|---|---|---|---|---|---|---|
| CRAB | PSAB | ST Allelic profiles | | IPM | MEM | *oxa-23* | *adeB* | | |
| 17 | 0 | ST92 1-3-3-2-2-7-3 | A | ≥32 | ≥32 | + | + | 2009 | SICU, RCU, Geriatrics, Emergency, Neurosurgery |
| 7 | 0 | ST92 1-3-3-2-2-7-3 | A | ≤4 | 8-16 | - | + | 2004-2006 | SICU , RCU |
| 7 | 0 | ST191 1-3-3-2-2-94-3 | A | ≥32 | ≥32 | + | + | 2009 | SICU |
| 5 | 0 | ST75 1-3-3-2-2-11-3 | A(4), B(1) | ≥32 | ≥32 | + | + | 2009 | RCU, Dermatology, Respiratory |
| 4 | 0 | STn1 6-n1-111-7-39-n2-11 | B | 16-32 | 16-32 | + | - | 2004 | PICU |
| 2 | 3 | ST17 1-12-12-11-4-103-3 | A(3), D(2) | ≤4 | ≤16 | - | + | 2007-2009 | SICU, Nephrology, RCU, Hematology |
| 2 | 0 | ST118 1-3-3-2-2-3-3 | A | ≥32 | ≥32 | + | + | 2009 | SICU |
| 1 | 0 | STn17 1-3-15-2-2-94-3 | A | ≥32 | ≥32 | + | + | 2009 | General surgery |
| 1 | 0 | STn4 8-3-111-52-3-n3 | A | 16 | 16 | - | + | 2007 | Respiratory |
| 1 | 0 | STn11 6-3-111-52-6-n11-65 | F | 16 | 16 | + | + | 2009 | Hematology |
| 0 | 2 | STn3 1-17-82-39-1-7-7 | C | ≤1 | ≤1 | - | + | 2005 | SICU |
| 0 | 1 | STn2 33-52-106-54-1-56-8 | C | ≤1 | ≤1 | - | + | 2004 | RCU |
| 0 | 1 | STn5 25-n4-n5-20-25-118-55 | H | ≤1 | ≤1 | - | - | 2009 | Respiratory |
| 0 | 1 | STn6 26-n6-n7-20-25-n8-28 | E | ≤1 | ≤1 | - | - | 2009 | Nephrology |
| 0 | 1 | STn7 9-3-111-7-39-n3-10 | F | ≤1 | ≤1 | - | - | 2009 | Geriatrics |
| 0 | 1 | STn8 6-3-111-7-39-n3-11 | F | ≤1 | ≤1 | - | - | 2009 | Geriatrics |
| 0 | 1 | STn9 30-3-n9-52-39-n10-56 | G | ≤1 | ≤1 | - | - | 2009 | Gynecology |
| 0 | 1 | STn10 21-35-2-28-1-143-4 | H | ≤1 | ≤1 | - | - | 2009 | Neurosurgery |
| 0 | 1 | STn12 33-31-2-28-1-144-5 | F | ≤1 | ≤1 | - | - | 2009 | Neurology |
| 0 | 1 | STn13 n12-31-49-11-1-25-4 | G | ≤1 | ≤1 | - | - | 2009 | Cardiac surgery |
| 0 | 1 | STn14 9-3-111-7-39-n13-11 | H | ≤1 | ≤1 | - | - | 2009 | Pediatrics |
| 0 | 1 | STn15 1-72-12-11-4-54-3 | H | ≤1 | ≤1 | - | - | 2009 | Pediatrics |
| 0 | 1 | STn16 21-12-n14-28-22-143-4 | E | ≤1 | ≤1 | - | - | 2009 | PICU |
| 0 | 1 | STn18 1-1-65-45-33-102-3 | E | ≤1 | ≤1 | - | - | 2009 | SICU |

Abbreviations: Surgical Intensive Care Unit (SICU), Respiratory Intensive Care Unit (RCU), Pediatric Intensive Care Unit (PICU).

different wards of our hospital in 2009 were collected, and some representative isolates of CRAB and PSAB between 2004 and 2009 were also included (Table 1). In this study, we define PSAB as susceptible to routinely tested antibiotics by VITEK 2 and PHOENIX 100 system. In order to exclude isolates affected by colonization, we chose CRAB and PSAB from specimens of blood, sterile body site or deep sputum samples (with leukocytes>25/HP, squamous epithelial cells <10/HP).

Bacteria identification was performed by VITEK-2 (bioMérieux, France) and PHOENIX-100 (BD, USA) automated system, and confirmed to be *A. baumannii* by *gyrB* multiplex (Higgins *et al*., 2007). All selected isolates were stored at -70°C and grown overnight on blood agar plates at 35°C before use.

### Antimicrobial susceptibility testing and detection of carbapenem resistance related genes

Antimicrobial susceptibility was tested by VITEK-2 and PHOENIX-100 automated system. The minimal inhibitory concentrations (MICs) of imipenem (IPM) and meropenem (MEM) were confirmed by Etest (AB Biodisk, Sweden). Results were interpreted according to the breakpoints from Clinical and Laboratory Standards Institute (CLSI, 2011). *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as quality controls in each set of tests.

Detection of carbapenemase genes (*oxa-23*, *oxa-24*, *oxa-51*, *oxa-58*, *imp*, and *vim*) and AdeABC efflux pump gene adeB in CRAB isolates were performed by Polymerase Chain Reaction (PCR) method as described previously (Huang *et al*., 2008).

### MLST

Multilocus sequence typing (MLST) was performed as described previously (Bartual *et al*., 2005), with some primers modified to get specific PCR products and satisfactory sequencing results (Fu *et al*., 2010). The annealing temperature of PCR amplification used in this study was 55°C for *gltA*, *gyrB*, *recA*, and *cpn60*, and 50°C for *gdhB*, *gpi*, and *rpoD*. The amplification products were purified with Qiagen DNA purification kit (Qiagen, USA). DNA sequencing was performed by ABI PRISM 3730 DNA analyzer (Applied Biosystems, USA). The allele sequences of 7 housekeeping genes were compared in Pubmlst database (http://pubmlst.org/abaumannii/) developed by Keith Jolley, then Sequence Types (STs) were designated according to the allelic profiles. eBURST (version 3, http://eburst.mlst.net/) was used to as-

sess the genetic relatedness of different STs as previously described (Feil *et al*., 2004).

### Rep-PCR

Amplification reaction was performed in a final volume of 50 μl. The primer pair was REP1: 5′-IIIGCGCCGICATCA GGC-3′, REP2: 5′-ACGTCTTATCAGGCCTAC-3′. The reaction conditions were described previously (Bou and Martinez-Beltran, 2000). Strains belonging to the same type should show identical or highly similar profiles (up to 2 bands difference).

## Results

### Characterization and antimicrobial susceptibility of included isolates

A total of 65 *A. baumannii* isolates, including 46 CRAB isolates and 19 PSAB isolates were selected between 2004 and 2009 from different wards of our hospital. The characteristics of these isolates were summarized in Table 1. Besides resistant to carbapenem, the CRAB isolates were also resistant to the following antibiotics: amikacin, ciprofloxacin, gentamicin, tetracycline, piperacillin, levofloxacin, cefepime, cefotaxime, ceftazidime, sulfamethoxazole/trimethoprim, cefoperazone/sulbactam, piperacillin/tazobactam, ampicillin/sulbactam, and minocycline. These CRAB isolates were only susceptible to polymycin E. The PSAB isolates were susceptible to all antibiotics mentioned above.

CRAB mainly resided in ICUs, and PSAB distributed in different wards. The CRAB ST92 isolates in 2009 were highly resistant to carbapenems, with $MIC_{IPM}$>32 mg/L and $MIC_{MEM}$ >32 mg/L, while the CRAB ST92 isolates in 2004 were intermediate or moderately resistant to carbapenems, with $MIC_{IPM}$≤4 mg/L and 8 mg/L≤$MIC_{MEM}$≤16 mg/L (Table 1).

### Detection of carbapenem resistance related genes

All included isolates in our study were *oxa-51* positive. The CRAB isolates in 2009 were *oxa-23* and *adeB* positive (Table 1). Other carbapenemase genes (*oxa-24*, *oxa-58*, *imp*, and *vim*) were negative in these isolates.

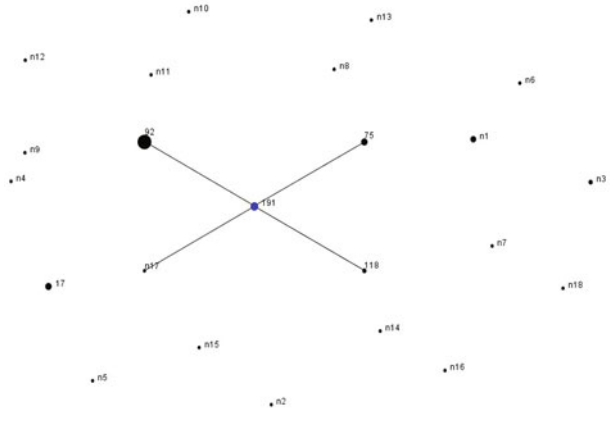


**Fig. 2. Population snapshot of *A. baumannii* in this study and existing isolates in Pubmlst database by eBURST algorithm.**

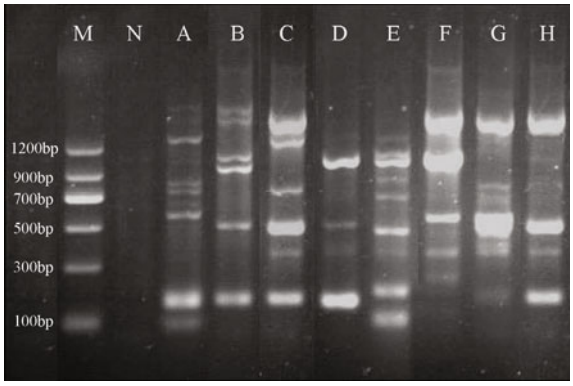


**Fig. 3. Gel picture of rep-PCR typing of each representative clone.** Lanes: M, molecular weight marker; N, negative control; A–H, typing pattern of each representative clone.

### MLST typing results

The MLST typing patterns of included isolates were summarized in Table 1. The novel unassigned alleles were consecutively numbered n1, n2, n3, etc. Their corresponding STs were consecutively numbered STn1, STn2, STn3, etc. The 65 included isolates were separated into 23 STs, and ST92 was the most frequent ST detected. Then eBURST algorithm was used with default parameter. 39 isolates belonging to ST191 (7/39), ST92 (24/39), ST75 (5/39), ST118 (2/39), and STn17 (1/39) respectively were clustered into a group, with ST191 being predicted founder. Other 26 isolates belonged to 18 singletons. PSAB clustered into more singletons than CRAB. The population snapshot of these isolates was presented in Fig. 2.

### Rep-PCR typing results

The gel picture of rep-PCR typing of each representative clone (A-H) was presented in Fig. 3, and typing results were summarized in Table 1. By rep-PCR, type A represented the major clone, which corresponded to ST92 and their single locus variants (SLVs) ST191, ST75, and ST118 and STn17. Other typing patterns represented different sporadic clones.

## Discussion

Both MLST and rep-PCR were used for molecular typing in the present study. MLST is a powerful tool for molecular epidemiologic study by putting the data into global context via internet (http://pubmlst.org/abaumannii/), thus comparison of data from different laboratories is realized. Rep-PCR is chosen for its ease of use, high throughput and its discriminatory power which is comparable to PFGE (Saeed *et al*., 2006). Generally, the results of MLST correlated well with rep-PCR (Table 1).

In this study, we examined the molecular epidemiology of CRAB and PSAB in our hospital during a 6-year period. ST92 was reported to spread in western China (He *et al*., 2011), and it was the predominant clone of CRAB in our hospital from 2004 to 2009. Different sporadic clones were found in PSAB, most of which belonged to novel and un-

related STs. This suggests that the increase of CRAB isolates in our hospital is probably due to clonal spread of ST92, instead of evolving from PSAB. Repeated isolation of ST92 is possibly due to readmission of previously colonized patients, interhospital transfers, and its long-term persistence in hospital environment. Previous studies reported that CRAB ST92 persisted longitudinally in single location (Diancourt *et al*., 2010) or geographic region (Di Popolo *et al*., 2011), but without continuity in one hospital presented here.

CRAB mainly resided in ICUs, while PSAB distributed in different wards. Thus we presume that CRAB clones may be more adaptable than PSAB. *A. baumannii* ST92 clone is a successful nosocomial pathogen because of its adaptation to hospital environment and antibiotic resistance (Runnegar *et al*., 2010). CRAB ST92 was mainly isolated from China, Australia, United States etc. Our results are in accordance with previous reports (Fu *et al*., 2010; Zhang *et al*., 2010; Adams-Haduch *et al*., 2011). ST191 was identified as the predicted founder by eBURST analysis in this study, which was the SLV of ST92. ST191 was first isolated in 2007 from Norway (Karah *et al*., 2011), which was CRAB and belonged to European clone II. In this study, PSAB clustered into more singletons than CRAB. This is probably because PSAB is more susceptible to antibiotic and hospital environment than CRAB. It could not survive as longitudinally as CRAB, which leads to the rapid change of different PSAB clones.

Different mechanisms are involved in carbapenem resistance in *A. baumannii*. Carbapenemase gene *oxa-51*, which was positive in our included isolates, is ubiquitous in *A. baumannii*, and its role in carbapenem resistance remains uncertain (Merkier and Centron, 2006). Carbapenemase gene *oxa-23* disseminates worldwide and is also prevalent in China (Fu *et al*., 2010). AdeABC efflux pump in *A. baumannii* is responsible for resistance of several antibiotics including carbapenems, and *adeB* gene is essential for the efflux function (Marchand *et al*., 2004), thus *adeB* was screened in our isolates. The positivity of *oxa-23* and *adeB* correlate with high level carbapenem resistance of CRAB ST92 isolates in 2009. Further study is still needed to make the cause-and-effect relationship.

In this study, 7 isolates of CRAB ST92 in 2004 only resided in SICU, while 17 isolates of CRAB ST92 in 2009 spread in various wards. These isolates in 2009 seem to be more adaptable to hospital environment, highly resistant to carbapenems (with *oxa-23* and *adeB* positive) and spread more widely in different wards, which corresponds to the trait of international epidemic ST92 clone. So the possibility of acquiring blaOXA-23 gene was low. As our hospital is a 1500-bed tertiary teaching hospital in Beijing, China, with the average outpatient visits of 7000/day, the patient transfers from different hospitals and various areas are common. It is more likely to be the epidemic ST92 clone carrying *blaOXA-23* outside has been introduced and spread in our hospital, although the precise origin and time point for the introduction of this clone is unknown. In summary, these observations suggest that clonal spread of ST92 longitudinally is the possible reason for carbapenem resistance rate increase and correlate with high level carbapenem resistance in our hospital.

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## References

**Adams-Haduch, J.M., Onuoha, E.O., Bogdanovich, T., Tian, G.B., Marschall, J., Urban, C.M., Spellberg, B.J., Rhee, D., Halstead, D.C., and Pasculle, A.W.** 2011. Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. *J. Clin. Microbiol.* **49**, 3849–3854.

**Bartual, S.G., Seifert, H., Hippler, C., Luzon, M.A., Wisplinghoff, H., and Rodriguez-Valera, F.** 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *J. Clin. Microbiol.* **43**, 4382–4390.

**Bou, G. and Martinez-Beltran, J.** 2000. Cloning, nucleotide sequencing, and analysis of the gene encoding an AmpC beta-lactamase in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **44**, 428–432.

**CLSI.** 2011. Performance standards for antimicrobial susceptibility testing: twenty first informational supplement M100-S21. Clinical and Laboratory Standards Institute. Wayne, PA, USA.

**Di Popolo, A., Giannouli, M., Triassi, M., Brisse, S., and Zarrilli, R.** 2011. Molecular epidemiological investigation of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries with a multilocus sequence typing scheme. *Clin. Microbiol. Infect*. **17**, 197–201.

**Diancourt, L., Passet, V., Nemec, A., Dijkshoorn, L., and Brisse, S.** 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* **5**, e10034.

**Feil, E.J., Li, B.C., Aanensen, D.M., Hanage, W.P., and Spratt, B.G.** 2004. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J. Bacteriol.* **186**, 1518–1530.

**Fu, Y., Zhou, J., Zhou, H., Yang, Q., Wei, Z., Yu, Y., and Li, L.** 2010. Wide dissemination of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* clonal complex 22 in multiple cities of China. *J. Antimicrob. Chemother*. **65**, 644–650.

**He, C., Xie, Y., Fan, H., Kang, M., Tao, C., Zhang, R., Hu, Y., Chen, Z., and Wang, L.** 2011. Spread of imipenem-resistant *Acinetobacter baumannii* of European clone II in Western China. *Int. J. Antimicrob. Agents* **38**, 257–260.

**Higgins, P.G., Wisplinghoff, H., Krut, O., and Seifert, H.** 2007. A PCR-based method to differentiate between *Acinetobacter baumannii* and *Acinetobacter* genomic species 13TU. *Clin. Microbiol. Infect*. **13**, 1199–1201.

**Huang, L., Sun, L., Xu, G., and Xia, T.** 2008. Differential susceptibility to carbapenems due to the AdeABC efflux pump among nosocomial outbreak isolates of *Acinetobacter baumannii* in a Chinese hospital. *Diag. Microbiol. Infect. Dis*. **62**, 326–332.

**Jolley, K.A., Chan, M.S., and Maiden, M.C.** 2004. mlstdbNet – distributed multi-locus sequence typing (MLST) databases. *BMC Bioinform*. **5**, 86.

**Karah, N., Haldorsen, B., Hermansen, N.O., Tveten, Y., Ragnhildstveit, E., Skutlaberg, D.H., Tofteland, S., Sundsfjord, A., and Samuelsen, O.** 2011. Emergence of OXA-carbapenemase- and 16S rRNA methylase-producing international clones of *Acineto-*

*bacter baumannii* in Norway. *J. Med. Microbiol*. **60**, 515–521.

**Marchand, I., Damier-Piolle, L., Courvalin, P., and Lambert, T.** 2004. Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. *Antimicrob. Agents Chemother*. **48**, 3298–3304.

**Merkier, A.K. and Centron, D.** 2006. bla(OXA-51)-type beta-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. *Int. J. Antimicrob. Agents* **28**, 110–113.

**Munoz-Price, L.S. and Weinstein, R.A.** 2008. *Acinetobacter* infection. *N. Engl. J. Med*. **358**, 1271–1281.

**Peleg, A.Y., Seifert, H., and Paterson, D.L.** 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev*. **21**, 538–582.

**Runnegar, N., Sidjabat, H., Goh, H.M., Nimmo, G.R., Schembri, M.A., and Paterson, D.L.** 2010. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a single institution over a 10-year period. *J. Clin. Microbiol*. **48**, 4051–4056.

**Saeed, S., Fakih, M.G., Riederer, K., Shah, A.R., and Khatib, R.** 2006. Interinstitutional and intrainstitutional transmission of a strain of *Acinetobacter baumannii* detected by molecular analysis: comparison of pulsed-field gel electrophoresis and repetitive sequence-based polymerase chain reaction. *Infect. Control. Hosp. Epidemiol*. **27**, 981–983.

**Seifert, H., Dolzani, L., Bressan, R., van der Reijden, T., van Strijen, B., Stefanik, D., Heersma, H., and Dijkshoorn, L.** 2005. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J. Clin. Microbiol*. **43**, 4328–4335.

**van Dessel, H., Dijkshoorn, L., van der Reijden, T., Bakker, N., Paauw, A., van den Broek, P., Verhoef, J., and Brisse, S.** 2004. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. *Res. Microbiol*. **155**, 105–112.

**Zhang, J.P., Zhu, W., Tian, S.F., Chu, Y.Z., and Chen, B.Y.** 2010. Molecular characteristics and resistant mechanisms of imipenem-resistant *Acinetobacter baumannii* isolates in Shenyang, China. *J. Microbiol*. **48**, 689–694.