Clonal diversity of *Acinetobacter baumannii* clinical isolates revealed by a snapshot study

Xiaohui Wang¹,², Fu Qiao³, Rujia Yu¹,², Yanyu Gao¹,² and Zhiyong Zong¹,²,³*

**Abstract**

**Background:** *Acinetobacter baumannii* is a notorious opportunistic pathogen mainly associated with hospital-acquired infections. Studies on the clonal relatedness of isolates could lay the foundation for effective infection control. A snapshot study was performed to investigate the clonal relatedness of *A. baumannii* clinical isolates in our local settings.

**Results:** Among 82 non-repetitive *Acinetobacter* spp. clinical isolates that were recovered during a period of four days in 13 hospitals in Sichuan, Southwest China, 67 isolates were identified as *A. baumannii*. Half of the 67 *A. baumannii* isolates were non-susceptible to carbapenems. *blaOXA-23* was the only acquired carbapenemase gene detected, present in 40 isolates including five carbapenem-susceptible ones. The isolates belonged to 62 pulsotypes determined by PFGE and 31 sequence types (ST) by multi-locus sequence typing. Forty-three isolates belonged to the globally-disseminated clonal complex 92, among which ST75, ST92 and ST208 were the most common sequence types.

**Conclusions:** Clinical isolates of *A. baumannii* were diverse in clonality in this snapshot study. However, most of the isolates belonged to the globally-distributed clonal complex CC92. ST75, ST92 and ST208 were the most common types in our region. In particular, ST208 might be an emerging lineage carrying *blaOXA-23*.

**Keywords:** Acinetobacter baumannii, Clonal relatedness, PFGE, MLST, *blaOXA-23*

**Background**

*Acinetobacter baumannii* is one of the common bacterial species responsible for hospital-acquired infections (HAIs) [1]. The prevalence of multi-drug resistant (MDR) *A. baumannii* in hospitals has been increasing worldwide [2], representing a serious challenge for clinical management and public health. Investigation on the clonal relatedness of *A. baumannii* in local settings could generate useful data to understand the local epidemiology of this opportunistic pathogen and therefore lay a foundation for an effective infection control program. Previous studies have focused on the clonal relatedness of *A. baumannii* but the vast majority of these studies were retrospective and used a collection of isolates either from outbreaks or with little information on their representativeness. For hospitals in Sichuan, Southwest China, *A. baumannii* was a huge problem as it was the most common bacterial species associated with HAIs and accounted for 17.3% of putative pathogens causing HAIs in a point prevalence survey [3]. Outbreaks due to *A. baumannii* had also been reported in our hospitals [4]. A snapshot study was therefore performed to investigate the clonal relatedness of *A. baumannii* clinical isolates in our local settings.

**Results and discussion**

Among 82 non-repetitive isolates that were recovered from clinical specimens from June 22 to June 25, 2011 in 13 hospitals in Sichuan and were putatively identified as *A. baumannii* by automated microbiology systems, 67 isolates were validated to be *A. baumannii*. The vast majority (61/67, 91%) of the *A. baumannii* isolates were recovered from sputa or respiratory tract secretions. The remaining six isolates were from ascites, cerebrospinal fluid, drainage, pleural fluid or wound secretions. As for the clinical significance, *A. baumannii* isolates were considered as either colonizers (55.2%) or pathogens (44.8%) causing clinical infections.
About half of the *A. baumannii* isolates (35/67, 52.2%) were non-susceptible to carbapenems (34 non-susceptible to both imipenem and meropenem and 1 non-susceptible to meropenem only), which was in consistency with the 53% carbapenem resistance rate of *A. baumannii* in the 2010 report of Chinese Ministry of Health National Antimicrobial Resistance Investigation Net (MOHNARIN) [5]. Many isolates were non-susceptible to sulbactam (35/67, 52.2%), ceftazidime (39/67, 58.2%), ciprofloxacin (43/67, 64.2%) or cotrimoxazole (47/67, 70.1%) while all isolates were susceptible to polymyxin and rifampicin and only one isolate was non-susceptible to minocycline.

bla<sub>OXA-23</sub> was the only acquired carbapenemase gene that was detected. Interestingly, it was present in 35/35 carbapenem-non-susceptible and 5/32 carbapenem-susceptible isolates. bla<sub>OXA-23</sub> has been the most common carbapenemase gene in China, as a previous study reported that 322 out of 342 (94.2%) imipenem-non-susceptible isolates belonged to 545 STs, the 305-bp *gpi* locus of *A. baumannii* appeared to diverge much faster than other loci, with 149 *gpi* allele types comparing to 45 *gltA*, 84 *gyrB*, 85 *gdiB*, 53 *recA*, 42 *cpn60* and 63 *rpoD*. The occurrence of a different *gpi* allele for every three STs deposited in the pubmlst database raised the concern that *gpi* might not be an ideal locus for typing. A previous study [8] also suggested that *gpi* was not a good candidate for MLST analysis due to recombination. Therefore, the diversity of *A. baumannii* generated by variations in the *gpi* locus alone might need validation to appreciate whether the diversity is truly meaningful.

Isolates belonging to each of 19 new STs were assigned to 18 STs using the Pasteur scheme (Table 1). Among the 18 STs determined by the Pasteur scheme, 12 had not been seen before and therefore were truly new STs. Although 411 STs of *A. baumannii* had been assigned in the pubmlst database before this study, a few new STs were still detected in a relatively small collection, suggesting that *A. baumannii* is likely to be extremely diverse in clonal origins or is undergoing a significant clonal expansion. Of note, the isolates with new STs were all carbapenem-susceptible except one. bla<sub>OXA-23</sub> was not detected in most (17/21) isolates of the novel STs. This phenomenon was also present in this study as all the local carbapenem-resistant isolates carrying bla<sub>OXA-23</sub> belonging to CC92. It has been suggested that among carbapenem-resistant isolates some belonging to certain clonal complexes appeared to be more successful [12-14].

The diversity of *A. baumannii* isolates in our settings could provide useful information for infection control. The clonal diversity of *A. baumannii* and the fact that carbapenem resistance could be transmitted horizontally highlight that "horizontal" infection control measures such as environmental cleaning and hand hygiene should be reinforced to reduce the further spread of *A. baumannii*. Person-to-person transmission of carbapenem-non-susceptible *A. baumannii* carrying bla<sub>OXA-23</sub> was indeed identified for several cases as evidenced by the fact that
**Figure 1** PFGE patterns of *A. baumannii* isolates. Dendrogram was generated by BioNumerics software with the unweighted pair-group method using arithmetic averages (UPGMA). Isolate name, ST, CC and the carriage of *bla*OXA-23 (Y, positive; N, negative) are indicated. The ST numbers shown after slash are assigned using the Pasteur MLST scheme.
| ST  | ST profile | CC  | Isolates no. | Hospital | PFGE types | No. isolates carrying blaOXA-23 | No. carbapenem-non-susceptible isolates |
|-----|------------|-----|--------------|----------|------------|-------------------------------|---------------------------------------|
| 17  | 1-12-12-11-4-10-3 | 17  | 2            | WC       | 3, 36      | 1                             | 0                                      |
| 75  | 1-3-3-2-2-11-3 | 92  | 6            | DZ, MY, WC | 4, 24, 26, 37, 50, 51 | 5                             | 4                                      |
| 92  | 1-3-3-2-2-7-3 | 92  | 8            | DZ, MY, NC, WC | 2, 8, 28, 34, 41, 47, 49, 56 | 6                             | 6                                      |
| 118 | 1-3-3-2-2-7-3 | 92  | 2            | MY       | 43, 44     | 2                             | 2                                      |
| 136 | 1-3-3-2-2-6-3 | 92  | 1            | WC       | 57         | 0                             | 0                                      |
| 138 | 1-3-3-2-2-50-3 | 92  | 3            | WC       | 25, 29, 61 | 1                             | 1                                      |
| 184 | 36-31-59-28-4-54-4 | 5   | 1            | SN       | 12         | 0                             | 0                                      |
| 191 | 1-3-3-2-2-94-3 | 92  | 2            | WC       | 33, 45     | 2                             | 2                                      |
| 208 | 1-3-3-2-2-97-3 | 92  | 13           | LS, LZ, MY, WC, YA | 20 (2), 21, 22, 23, 27, 38, 39, 40, 46, 48, 49, 59 | 12                             | 12                                     |
| 218 | 1-3-3-2-2-102-3 | 92  | 2            | MY       | 42, 44     | 2                             | 2                                      |
| 368 | 1-3-3-2-2-140-3 | 92  | 2            | MY, WC   | 24, 60     | 2                             | 2                                      |
| 381 | 1-81-3-3-2-2-16-3 | 92  | 3            | LE, PZ   | 1, 18 (2)  | 3                             | 3                                      |
| 427/40 | 1-31-12-4-25-3 | 17/216 | 2       | WC       | 15, 62     | 2                             | 0                                      |
| 428/33 | 21-15-2-28-1-52-4 | 145/33 | 1   | WC       | 54         | 0                             | 0                                      |
| 429/332 | 1-34-56-1-144-45 | S/216 | 2         | MS, WC   | 5, 14      | 0                             | 0                                      |
| 430/333 | 1-31-49-10-1-103-4 | S/5 | 1         | WC       | 30         | 0                             | 0                                      |
| 431/334 | 34-12-68-41-1-104-4 | 80/34 | 1         | WC       | 55         | 0                             | 0                                      |
| 432/34 | 34-12-68-41-32-104-4 | 80/34 | 1         | NC       | 32         | 0                             | 0                                      |
| 433/2 | 1-81-3-1-2-16-3 | 92/2 | 1         | PZ       | 19         | 1                             | 1                                      |
| 475/113 | 18-44-2-28-1-151-71 | S/113 | 1         | WC       | 35         | 0                             | 0                                      |
| 476/341 | 35-50-42-49-48-157-5 | S/S | 1         | WC       | 31         | 0                             | 0                                      |
| 477/268 | 1-15-80-1-6-9-36-26 | S/216 | 1         | ZG       | 9          | 0                             | 0                                      |
| 478/339 | 33-89-40-26-32-91-5 | 163/5 | 1         | WC       | 13         | 0                             | 0                                      |
| 479/331 | 52-39-80-5-1-161-12 | S/216 | 1         | WC       | 58         | 0                             | 0                                      |
| 480/331 | 1-34-80-28-1-160-45 | 172/216 | 1         | WC       | 7          | 0                             | 0                                      |
| 481/340 | 1-12-67-6-50-159-6 | S/243 | 1         | WC       | 6          | 0                             | 0                                      |
| 481/340 | 1-12-67-6-50-159-6 | S/243 | 1         | WC       | 6          | 0                             | 0                                      |
| 482/335 | 1-90-6-1-36-102-6 | S/216 | 1         | NC       | 17         | 0                             | 0                                      |
| 483/342 | 24-1-129-11-49-16-26 | S/158 | 1         | PZ       | 52         | 0                             | 0                                      |
| ST   | Profile         | CC | Hospital       | Number |
|------|----------------|----|----------------|--------|
| 484/337 | 1-91-130-1-36-110-72 | 1  | DZ             | 16     |
| 485/338 | 33-12-59-11-32-158-5  | 5  | DY             | 10     |
| 503/336 | 1-62-80-28-35-164-45 | 1  | LZ             | 11     |
| ND/203 | 1-34-ND-28-1-165-45 /216 | 1  | WC             | 53     |

1ND, not determined. For new STs (ST427 and above) and the undetermined ST by the pubmlst scheme, STs determined using the Pasteur MLST scheme are shown after the slash “/” with new STs being underlined.

2CC, clonal complex. S, singleton. CCs for STs determined using the Pasteur MLST scheme are shown after the slash “/”. The members of CCs for STs determined using the pubmlst scheme in Table 1 other than CC92 are listed as below. CC17 (n = 6): ST17, ST373, ST427, ST528, ST530, ST554; CC80 (n = 4): ST80, ST360, ST431, ST432; CC145 (n = 11): ST85, ST86, ST144, ST145, ST153, ST158, ST376, ST401, ST428, ST552, ST553; CC163 (n = 8): ST163, ST164, ST165, ST166, ST167, ST458, ST478, ST574; CC172 (n = 3): ST172, ST412, ST480; CC334 (n = 2): ST334, ST485.

3Hospital: DY, Deyang People’s Hospital, Deyang city; DZ, Dazhou Central Hospital, Dazhou city; LE, Leshan People’s Hospital, Leshan city; LS, The First Hospital of Liangshan Prefecture, Xichang city; LZ, Affiliated Hospital of Luzhou Medical College, Luzhou city; MS, Meishan People’s Hospital, Meishan City; MY, Mianyang Central Hospital, Mianyang city; NC, Affiliated Hospital of North Sichuan Medical College, Nanchong city; PZ, Panzhihua Central Hospital, Panzhihua city; SN, Suining Central Hospital, Suining city; WC, West China Hospital of Sichuan University, Chengdu city; YA, Yaan People’s Hospital, Yaan city; ZG, The First Municipal Hospital of Zigong, Zigong city.

4The number in brackets indicates the number of isolates belonging to the same pulsotype. Isolates of the same pulsotype were recovered from different patients in the same hospital.
isolates recovered from different patients belonged to the same pulsotype (Table 1 and Figure 1). This suggests that effective infection control measures might need to include rapid identification of \textit{bla}_{OXA-23} by molecular methods and also justifies contact precautions for patients with carbapenem-resistant isolates.

**Figure 2** STs seen in this study and the CC92. Panel A. The relatedness of the STs seen in this study. Panel B. The clonal complex 92 (CC92). eBURST was performed using 6 as the minimum identical loci for the definition of CC and 3 as the minimum single locus variants. The countries of isolation are shown according to the pubmlst database. The STs found in this study are in bold. NA, information not available. ST208 is predicted as the founder ST for CC92 by eBURST.

**Conclusions**
This study provided a snapshot of \textit{A. baumannii} population in clinical samples in our local settings. Significantly diverse clonal origins were identified but most isolates belonged to the globally-distributed CC92. Among CC92, ST75, ST92 and ST208 were the most common
types in our region. The high prevalence of ST208 carrying \textit{bla}_{OXA-23} suggests that ST208 appears to be an emerging lineage mediating the spread of carbapenem resistance. The diversity of \textit{A. baumannii} suggested that the current MLST scheme might need to be further optimized and in particular the \textit{gpi} gene might not be an ideal target for \textit{Acinetobacter} MLST.

**Methods**

**Strains**
The study included all non-repetitive isolates (n = 82) that were recovered from clinical specimens from June 22 to June 25, 2011 in 13 hospitals in Sichuan, southwest China and were putatively identified as \textit{A. baumannii} or belonging to the \textit{Acinetobacter calcoaceticus-baumannii} complex using the Vitek II, MicroScan and Phoenix automated systems. The clinical samples were taken as part of standard patient care and therefore no ethical approval was applied for their use. The 13 hospitals are all tertiary with 19,051 beds in total (ranged from 800 to 4,300) including 3 university hospitals and 10 municipal ones. For each patient, only one isolate was collected. Genomic species identification was established by partially sequencing the \textit{recA} gene as described previously [15].

**In vitro susceptibility test**
MICs of meropenem, imipenem, ceftazidime, sulbactam, minocycline, polymyxin, ciprofloxacin, rifampicin and cotrimoxazole against \textit{A. baumannii} isolates were determined using the agar dilution method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [16]. MICs were interpreted according to the breakpoints established by CLSI [16], except for sulbactam and rifampicin, for which breakpoints from the French Society for Microbiology were used (for sulbactam, ≤8 mg/L for susceptible; for rifampicin, ≤8 µg/ml for susceptible and >16 mg/L for resistant) [17]. Resistance to imipenem or meropenem was defined as carbapenem resistance.

**Detection of carbapenemase-encoding genes**
Genes encoding Class A carbapenemases (\textit{bla}_{GES} and \textit{bla}_{KPC}), Class B metallo-\(\beta\)-lactamases (\textit{bla}_{IMB}, \textit{bla}_{VIM}, \textit{bla}_{SPM}, \textit{bla}_{GMB}, \textit{bla}_{SIM} and \textit{bla}_{NDM}) or Class D OXA-type carbapenemases (\textit{bla}_{OXA-51}, \textit{bla}_{OXA-23}, \textit{bla}_{OXA-24}, \textit{bla}_{OXA-58} and \textit{bla}_{OXA-143}) were screened as described previously [18-22]. Purified amplicons were sequenced in both directions using an ABI 3730 DNA analyzer (Applied Biosystems, Warrington, United Kingdom). Similarity searches were carried out using BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST/).

**Strain typing**
PFGE was employed to determine clonal relatedness of the isolates and was performed as described previously [12]. PFGE band patterns were analyzed using the BioNumerics software, version 6.6.4.0 (Applied Mathys, St-Martens-Latem, Belgium). Pulsotypes were defined as isolates with PFGE band patterns of 80% similarity or above [23].

All \textit{A. baumannii} isolates were subjected to MLST targeting seven housekeeping genes, \textit{gltA}, \textit{gyrB}, \textit{gdiB}, \textit{recA}, \textit{cpm60}, \textit{gpi} and \textit{rpoD} [24]. As primers used previously were unable to amplify the \textit{gdlhB} and \textit{gpi} alleles for some isolates [9,24,25], new primers were therefore designed for \textit{gdiB} (\textit{gdlhBxR1}: ATTTGTTTGCTGCGGAATAGT; \textit{gdlhBxR1}: TA TGGGGGCCAGATAAATCAA) and \textit{gpi} (\textit{gpi-F2}: AAA ATCCATGCTGGGCAATA; \textit{gpi-R2}: CCGAGTAATGCC ATGAGAAC) genes [24]. New STs were deposited in the \textit{Acinetobacter} MLST database (http://pubmlst.org/abumannii/). eBURST (version 3, http://eburst.mlst.net/) was used to assign STs to CCs, which were defined for those sharing identical alleles at six of seven loci. CCs were named according to the number of the predicted founder ST except for CC92, which has been well defined in literature. If no founder ST was predicted by eBURST, the CC was named by the first ST assigned.

Isolates with new STs and isolate d34, of which ST could not be determined using the pubmlst scheme, were also subjected to MLST using the Pasteur scheme [26]. New STs determined using the Pasteur scheme have also been deposited into the database (http://www.pasteur.fr/mlst/Abumannii.html).

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
WX carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. QF carried out the species targeting seven housekeeping genes, \textit{gltA}, \textit{gyrB}, \textit{gdiB}, \textit{recA}, \textit{cpm60}, \textit{gpi} and \textit{rpoD} [24]. As primers used previously were unable to amplify the \textit{gdlhB} and \textit{gpi} alleles for some isolates [9,24,25], new primers were therefore designed for \textit{gdiB} (\textit{gdlhBxR1}: ATTTGTTTGCTGCGGAATAGT; \textit{gdlhBxR1}: TA TGGGGGCCAGATAAATCAA) and \textit{gpi} (\textit{gpi-F2}: AAA ATCCATGCTGGGCAATA; \textit{gpi-R2}: CCGAGTAATGCC ATGAGAAC) genes [24]. New STs were deposited in the \textit{Acinetobacter} MLST database (http://pubmlst.org/abumannii/). eBURST (version 3, http://eburst.mlst.net/) was used to assign STs to CCs, which were defined for those sharing identical alleles at six of seven loci. CCs were named according to the number of the predicted founder ST except for CC92, which has been well defined in literature. If no founder ST was predicted by eBURST, the CC was named by the first ST assigned.

Isolates with new STs and isolate d34, of which ST could not be determined using the pubmlst scheme, were also subjected to MLST using the Pasteur scheme [26]. New STs determined using the Pasteur scheme have also been deposited into the database (http://www.pasteur.fr/mlst/Abumannii.html).

**Acknowledgments**
This work was partially supported by a grant from China US Collaborative Program on Emerging and Reemerging Infectious Diseases and by a grant from the National Natural Science Foundation of China (project no. 81101293). The authors declare that they have no competing interests.

**Authors’ contributions**
WX carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. QF carried out the species targeting seven housekeeping genes, \textit{gltA}, \textit{gyrB}, \textit{gdiB}, \textit{recA}, \textit{cpm60}, \textit{gpi} and \textit{rpoD} [24]. As primers used previously were unable to amplify the \textit{gdlhB} and \textit{gpi} alleles for some isolates [9,24,25], new primers were therefore designed for \textit{gdiB} (\textit{gdlhBxR1}: ATTTGTTTGCTGCGGAATAGT; \textit{gdlhBxR1}: TA TGGGGGCCAGATAAATCAA) and \textit{gpi} (\textit{gpi-F2}: AAA ATCCATGCTGGGCAATA; \textit{gpi-R2}: CCGAGTAATGCC ATGAGAAC) genes [24]. New STs were deposited in the \textit{Acinetobacter} MLST database (http://pubmlst.org/abumannii/). eBURST (version 3, http://eburst.mlst.net/) was used to assign STs to CCs, which were defined for those sharing identical alleles at six of seven loci. CCs were named according to the number of the predicted founder ST except for CC92, which has been well defined in literature. If no founder ST was predicted by eBURST, the CC was named by the first ST assigned.

Isolates with new STs and isolate d34, of which ST could not be determined using the pubmlst scheme, were also subjected to MLST using the Pasteur scheme [26]. New STs determined using the Pasteur scheme have also been deposited into the database (http://www.pasteur.fr/mlst/Abumannii.html).

**References**
1. Peleg AY, Seifert H, Paterson DL. \textit{Acinetobacter baumannii: emergence of a successful pathogen}. Clin Microbiol Rev 2008, 21:538-582.
2. Dijkstra, L, Nemec, A, Seifert, H: An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 2007, 5:393–951.

3. Zong, Z, Qiao, F, Yin, W, Xu, S: A large-scale survey on the point prevalence of healthcare-associated infections in southwest China. In IDWeek. San Diego, CA; Poster Nr: 2012:1711.

4. Zong, Z, Lu, X, Valenzuela, JF, Parmelee, SR, Iedell, J: An outbreak of carbapenem-resistant Acinetobacter baumannii producing OXA-23 carbapenemase in western China. Int J Antimicrob Agents 2008, 31:50–54.

5. Li, Y, Lu, Y, Wang, S: Mohnarin report 2010: surveillance of antimicrobial resistance in nonfermenting gram-negative bacteria. Chin J Nosocomiol 2011, 21:5133–5137 (behalf of Mohnarin).

6. 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.

7. Wang, X, Zong, Z, Lu, X: In 2008 a major vehicle carrying blaOXA-23 in Acinetobacter baumannii from China. Diagn Microbiol Infect Dis 2011, 69:218–222.

8. Hamouda, A, Evans, BA, Towner, KJ, Armes, SG: Characterization of epidemiologically unrelated Acinetobacter baumannii isolates from four continents by use of multilocus sequence typing, pulsed-field gel electrophoresis, and sequence-based typing of blaOXA-51-like genes. J Clin Microbiol 2010, 48:2476–2483.

9. Fu, Y, Zhou, J, Zhou, H, Yang, Q, Wei, Z, Yu, Y, Li, L: 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.

10. Adams-Haduch, JM, Onoucha, EQ, Bogdanovich, T, Tian, GB, Marschall, J, Urban CM, Spellberg, BJ, Rhee, D, Halstead, DC, Pasculle, AW, et al.: Molecular epidemiology of carbapenem-non-susceptible Acinetobacter baumannii in the United States. J Clin Microbiol 2011, 49:3849–3854.

11. Mugnier, PD, Pozega, N, Basser, S, Nordmann, P: Worldwide dissemination of the blaOXA-23 carbapenemase gene of Acinetobacter baumannii. Emerg Infect Dis 2010, 16:35–40.

12. Seifert, H, Dolzani, L, Bressan, R, van der Reijden, T, Van Strijen, B, Stefanik, D, Heems, H, Dijkstra, L: Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of Acinetobacter baumannii. J Clin Microbiol 2005, 43:4326–4335.

13. Karah, N, Sundsfjord, A, Towner, K, Samuelsen, O: Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. Drug Resist Updat 2012, 15:237–247.

14. Zanelli, R, Pournaras, S, Giannouli, M, Tzikas, A: Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages. Int J Antimicrob Agents 2013, 41:11–19.

15. Krawczyk, B, Lewandowski, K, Kur, J: Comparative studies of the Acinetobacter genus and the species identification method based on the recA sequences. Mol Cell Probes 2002, 16:11–11.

16. CSLi: Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement. Wayne, PA: The Clinical and Laboratory Standards Institute; 2011.

17. Comité de l’antibiogramme de la Societé Française de Microbiologie: Communiqué. Paris, France: Societé Française de Microbiologie; 2009.

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

19. Higgens, 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.

20. 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.

21. Pozega, N, Le, Thomas, I, Naas, T, Karim, A, Nordmann, P: Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from Klebsiella pneumoniae. Antimicrob Agents Chemother 2000, 44:622–632.

22. Bradford, PA, Bratu, S, Urban, C, Visalli, M, Mariano, N, Landman, D, Rahal JJ, Brooks, C, Cebular, S, Quale, J: Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 β-lactamases in New York City. Clin Infect Dis 2004, 39:55–60.

23. Van Belkum, A, Tassiou, PT, Dijkstra, H, Haeggman, S, Cookson, B, Fung, NYK, Fussing, V, Green, J, Feil, E, Gerner-Smidt, P, et al. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol Infect 2007, 13(Suppl 1):46–46.

24. Baruah, 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.

25. Hamidian, M, Hall, RM: Abar4 replaces Abar3 in a carbapenem-resistant Acinetobacter baumannii isolate belonging to global clone 1 from an Australian hospital. J Antimicrob Chemother 2011, 66:2484–2491.

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