Molecular epidemiology of Acinetobacter baumannii in Iran: endemic and epidemic spread of multiresistant isolates

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Objectives: We examined the molecular epidemiology of Acinetobacter baumannii clinical isolates from two cities (Tehran and Tabriz) of Iran.

Methods: DiversiLab repetitive extragenic palindromic PCR (rep-PCR), multilocus sequence typing and sequence group multiplex PCR were performed. The presence of resistance mechanisms including metallo-β-lactamases, extended-spectrum β-lactamases, OXA carbapenemases, aminoglycoside-modifying enzymes and RNA methylases was also investigated.

Results: DiversiLab rep-PCR identified 11 clusters and 11 singleton isolates. Twelve sequence types (STs), including six novel types, were identified. Sequence groups (SGs) 1–3 as well as five additional banding patterns were detected by multiplex PCR. A local outbreak in a general hospital in Tabriz with an SG1/ST2 profile was identified. Isolates of international clone II showed the highest prevalence and the most heterogeneous combination of resistance determinants.

Conclusions: Several different multiresistant strains of A. baumannii were shown to circulate in Iran. The selection and spread of the SG1/ST2 clone might have been favoured by the acquisition of resistance genes in the absence of adequate infection control measures.

Keywords: multidrug resistance, DiversiLab rep-PCR, MLST

Introduction

Acinetobacter baumannii is a major opportunistic pathogen in healthcare settings worldwide.1,2 The organism’s ability to spread among hospitalized patients and to persist for extended periods along with multi- or pan-drug resistance are the main driving forces behind the frequent large outbreaks in different countries.3–5 International clones (ICs) I, II and III have been found to be responsible for most outbreaks globally.6 Consequently, molecular typing is indispensable for tracing the sources and transmission routes of A. baumannii.7,8

Recently, DiversiLab repetitive extragenic palindromic PCR (rep-PCR) has been used as an alternative typing tool to PFGE. Indeed, rep-PCR has shown a comparable discriminatory power coupled with higher throughput and interlaboratory reproducibility than PFGE.9 An additional typing method, multilocus sequence typing (MLST), also offers the possibility of transferring typing data between laboratories, making it a more appropriate technique for global epidemiological studies.9

So far there are few reports on the clonality of A. baumannii isolates using DiversiLab rep-PCR and MLST in the Middle East.5,10,11 In Iran, to the best of our knowledge, the molecular epidemiology of A. baumannii isolates has not been previously reported using MLST. Herein, we report the results of the first comprehensive molecular epidemiological study of a set of isolates from Iran. To this end, DiversiLab rep-PCR was used as a preliminary typing approach. MLST, sequence group (SG) determination and characterization of the array of genes determining resistance were used to further characterize isolates at a clonal level.

Materials and methods

Bacterial isolates

A total of 71 consecutive non-duplicate clinical isolates of Acinetobacter spp. were collected in Iran from November 2010 to June 2011. Thirty-two isolates from a university-affiliated general hospital in Tabriz had been cultured from various clinical samples. A further 39 isolates
Table 1. Epidemiological, phenotypic and genotypic features of A. baumannii isolates included in this study

| Rep-PCR | ST No. | Tehran | Tabriz | OXA-23 | OXA-24 | TEM-1 | PER-1 | armA | aac(3′)-Ia | aac(6′)-Ib | aph(3′)-Ia | aph(3′)-Vla | ant(2′)-Ia | ant(3′)-Ia | IPM | MEM | CAZ | FEP | AMK | GEN | CIP |
|---------|-------|--------|--------|--------|--------|-------|-------|------|----------|----------|----------|----------|----------|----------|----------|-----|-----|-----|-----|-----|-----|-----|
| A       | 307   | 6      | 6      | 0      | 0      | 0     | 0     | 1    | 0        | 0        | 1        | 6        | 2        | 1        | 6       | 6   | 6   | 6   | 6   | 6   | 6   | 6   |
| B       | 25    | 2      | 2      | 0      | 0      | 2     | 0     | 2    | 0        | 0        | 0        | 2        | 2        | 2        | 0        | 2   | 2   | 2   | 2   | 2   | 2   | 2   |
| C       | 2     | 6      | 0      | 6      | 1      | 0     | 6     | 5    | 1        | 5        | 1        | 5        | 3        | 2        | 1        | 1   | 6   | 6   | 6   | 3   | 6   | 6   |
| C'      | 2     | 2      | 0      | 2      | 0      | 0     | 2     | 0    | 1        | 0        | 0        | 2        | 1        | 0        | 0        | 0   | 2   | 2   | 0   | 2   | 2   | 2   |
| J       | 2     | 11     | 3      | 8      | 11     | 1     | 2     | 6    | 2        | 6        | 2        | 3        | 10       | 1        | 1        | 11  | 11  | 11  | 11  | 11  | 11  | 11  |
| K       | 2     | 6      | 1      | 5      | 6      | 0     | 1     | 4    | 1        | 4        | 0        | 2        | 6        | 2        | 1        | 6   | 6   | 1   | 4   | 6   | 6   | 6   |
| Si 1,2  | 2     | 2      | 0      | 2      | 2      | 0     | 1     | 0    | 0        | 0        | 1        | 0        | 1        | 2        | 1        | 1   | 2   | 2   | 2   | 2   | 2   | 2   |
| D       | 85    | 3      | 1      | 2      | 3      | 1     | 0     | 0    | 0        | 0        | 3        | 1        | 0        | 3        | 1        | 0   | 3   | 3   | 3   | 3   | 3   | 3   |
| E       | 327   | 2      | 1      | 1      | 0      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| F       | 136   | 7      | 7      | 0      | 4      | 3      | 0     | 0    | 0        | 0        | 1        | 1        | 1        | 7        | 7        | 1    | 7   | 7   | 7   | 7   | 7   | 7   |
| F'      | 136   | 2      | 2      | 0      | 0      | 2      | 0     | 0    | 0        | 0        | 0        | 1        | 0        | 2        | 2        | 0    | 2   | 2   | 2   | 2   | 2   | 2   |
| Si 3    | 136   | 1      | 1      | 0      | 1      | 0      | 0     | 1    | 0        | 0        | 0        | 1        | 1        | 0        | 1        | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| G       | 328   | 7      | 7      | 0      | 7      | 7      | 0     | 0    | 0        | 1        | 2        | 1        | 6        | 6        | 1        | 7   | 7   | 7   | 7   | 7   | 7   | 7   |
| Si 4    | 328   | 1      | 1      | 0      | 1      | 1      | 0     | 0    | 0        | 0        | 1        | 0        | 1        | 1        | 0        | 1   | 1   | 1   | 1   | 1   | 1   | 1   |
| H       | 94    | 2      | 2      | 0      | 2      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 0        | 2        | 0        | 0   | 2   | 2   | 2   | 2   | 2   | 2   |
| I       | 323   | 3      | 1      | 2      | 3      | 0      | 0     | 0    | 0        | 0        | 1        | 0        | 3        | 3        | 2        | 3   | 3   | 3   | 3   | 3   | 3   | 3   |
| I'      | 323   | 1      | 1      | 0      | 1      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 0        | 1        | 1        | 0    | 1   | 1   | 2   | 1   | 2   | 2   |
| Si 6,9  | 323   | 2      | 1      | 1      | 2      | 0      | 0     | 0    | 0        | 0        | 0        | 1        | 1        | 2        | 2        | 2    | 2   | 2   | 2   | 2   | 2   | 2   |
| Si 7    | 323   | 1      | 0      | 1      | 0      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 1        | 1        | 1        | 1    | 1   | 1   | 1   | 1   | 1   | 1   |
| Si 11   | 324   | 1      | 1      | 0      | 1      | 1      | 0     | 0    | 0        | 0        | 0        | 0        | 1        | 0        | 1        | 0    | 1   | 1   | 1   | 1   | 1   | 1   |
| Si 8    | 325   | 1      | 1      | 0      | 1      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 1        | 1        | 0        | 1    | 1   | 1   | 1   | 1   | 1   | 1   |
| Si 5,10 | 326   | 2      | 0      | 2      | 0      | 0      | 0     | 0    | 0        | 0        | 0        | 0        | 0        | 0        | 0        | 0    | 0   | 0   | 0   | 0   | 0   | 0   |

*Different sequence group PCR banding patterns.
Si, singleton; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CAZ, ceftazidime; FEP, cefepime; CIP, ciprofloxacin.
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had been recovered from burn wound infections of hospitalized patients in the level I burn care centre in Tehran.

Microbiological methods

All isolates were identified by conventional biochemical techniques. Identification to the species level was performed by amplified ribosomal DNA restriction analysis and by species-specific PCR for the \( \text{bla}_{OXA-51} \)-like gene.

Antimicrobial susceptibility testing

The MICs of imipenem, meropenem (AB BIODISK, Solna, Sweden), ceftazidime, cefepime, ciprofloxacin, amikacin and gentamicin (Liofilchem, Italy) were determined using Etest strips. Results were interpreted in accordance with CLSI guidelines. Isolates showing intermediate levels of susceptibility were classified as non-susceptible.

Screening of resistance genes

PCR experiments were carried out using primers specific for the genes encoding IMP, VIM, SPM-1, PER-1, TEM-, VEB-1, SHV-, GES-1, CTX-M group 2, \( \text{bla}_{OXA-23} \)-like, \( \text{bla}_{OXA-40} \)-like, \( \text{bla}_{OXA-51} \)-like, \( \text{bla}_{OXA-68} \)-like and \( \text{bla}_{OXA-58} \). In addition, detection of \( \text{armA}, \text{mtb}, \text{aph}(3')-\text{Vla}, \text{aph}(3')-\text{Ia}, \text{aac}(6')-\text{Ib}, \text{aac}(3')-\text{Ia}, \text{aac}(3')-\text{IIa}, \text{ant}(3')-\text{Ia} \) and \( \text{ant}(2'\prime)-\text{Ia} \) genes was performed as described previously.

Molecular typing methods

rep-PCR was performed using the DiversiLab Acinetobacter kit. Results were analysed with the DiversiLab software as described previously using the modified Kullback–Leibler statistical method. Isolates that clustered at 95% similarity were considered related and defined rep-PCR clusters.

Genotyping by MLST was performed on representative isolates selected on the basis of clustering by rep-PCR, i.e. one isolate from each cluster and all singleton isolates. MLST was performed according to Nemec et al. Details of the MLST scheme are available at www.pasteur.fr/mlst. Sequence types (STs) were defined as single-locus variants (SLVs) and double-locus variants (DLVs) using eBURST V2 (http://eburst.mlst.net/default_v2.asp). Clonal complexes (CCs) were defined as containing at least three STs sharing the same allele numbers in at least six loci.

SG identification was conducted on all isolates as a screening method for SGs 1–3 using a multiplex PCR-based typing scheme as described previously.

Results

Overall, 67 out of 71 isolates (94.4%) were resistant to at least three classes of antibiotic and were considered multidrug resistant. The prevalence rate of resistance to most antibiotics was >75% (Table 1). In particular, 60 (84.5%) isolates were resistant to both carbapenems and amikacin, 65 (91.5%) were resistant to gentamicin and 67 (94.4%) were resistant to both ceftazidime and cefepime (Table 1).

The most prevalent resistance gene was \( \text{aph}(3')-\text{Vla} \) (n = 64), followed by \( \text{bla}_{OXA-23} \)-like (n = 47) and \( \text{ant}(2'\prime)-\text{Ia} \) (n = 38). Other
resistance determinants were detected in a proportion of isolates ranging between 33.8% and 8.4% (Table 1).

A total of 11 DiversiLab rep-PCR clusters (named A–K) were recognized, whereas 11 isolates were singletons (Figure 1). Twelve previously known STs were identified, including ST2, ST25, ST85, ST94, ST136 and ST307, and six novel STs: ST232 to ST328. Each ST corresponded to one rep-PCR cluster, except for ST2, which included heterogeneous rep-PCR profiles, i.e. clusters C, J and K, showing 48% similarity. Singleton 1, which also belonged to ST2, clustered at 84.1% similarity with isolates of cluster C.

Strains belonging to rep-PCR clusters A, B, F, G and H, and assigned to ST307, ST25, ST136, ST328 and ST94, respectively, were isolated in Tehran. Strains included in cluster C and attributed to ST2 were isolated in Tabriz only. The isolates included in the remaining clusters D, E and I originated from both cities and were assigned to ST85, ST327 and ST323, respectively. Isolates in clusters J and K, which were assigned to ST2, were also recovered from both cities (Table 1 and Figure 1).

Some STs were SLVs/DLVs of previously known STs. The eBURST analysis of the 12 STs, as compared with the 456 profiles of the database, showed that ST307 and ST325 were SLVs of STs 25 and 94, respectively. In addition, ST324 and ST328 proved to be DLVs of ST25 and ST1, respectively.

Multiplex PCR attributed 29, 9 and 7 isolates, respectively, to SGs 1, 2 and 3. The remaining 26 isolates showed 10 additional different patterns. The 29 isolates belonging to SG1 (IC II) were classified into three STs: ST2, ST85 and ST324, of which ST2 was the most prevalent type (21 isolates from Tabriz and 4 from Tehran). SG2 (IC I) included two new STs: ST328 (n=8) and ST323 (n=1). All ST328 isolates were from Tehran. Seven isolates of ST316 from Tehran showed a banding pattern corresponding to SG3.

Of the 60 carbapenem-resistant isolates, 40 (66.7%) proved to belong to ICs I and II. Isolates of IC I and IC II showed the most variable combinations of aminoglycoside-modifying enzymes (7 and 14, respectively). We also found three and nine different aminoglycoside-modifying enzyme combinations among isolates of IC III and isolates characterized by multiplex PCR banding patterns different from SGs 1–3.

Discussion

This study provides for the first time a comprehensive view of clonal diversity among A. baumannii in Iran obtained using different molecular typing methods.

High resistance rates were detected to most of the clinically available antimicrobial agents, including carbapenems. Carbapenem resistance appears mainly to be driven by acquired carbapenemases, namely blaOXA-23-like and blaOXA-40-like. Moreover, our observation that about 67% of carbapenem-resistant isolates belonged to epidemic ICs is consistent with previous studies showing that carbapenem resistance is strongly associated with the worldwide spread of these clones.17,19

In our study, IC II was the most common clone, in accordance with previous reports.8,10,17 Isolates of IC II also presented striking combinations of resistance genes, consistent with the IC II clone being a relatively older group that has been undergoing extensive diversification.3 Moreover, the IC II isolates showed the highest prevalence of most genetic determinants of resistance, supporting the view that the successful spread of IC II may have resulted from its selective advantage in the ‘antibiotic-rich’ hospital environment. The attribution of ST136 to SG3 (IC III) was inconsistent with previous reports. However, the unreliability of the PCR multiplex sequence grouping has previously been reported.20

The number of different STs, of which six were previously known and six were newly recognized, was to some extent unexpected, because our study was limited to two hospital settings in different Iranian cities. Moreover, the range of STs in Tehran was larger than in Tabriz (11 versus 5 STs), as a likely consequence of the admission to the Tehran burn unit, a specialized reference centre, of severely injured patients from all over the country. It can be also noted that two clusters of isolates identified in Tehran were attributed to ST328, a DLV of ST1, and with ST307, an SLV of ST25, a worldwide spreading multiresistant clone.19 Of further interest, clonal dissemination of ST2/SG1 strains heavily predominated in different wards of a general hospital in Tabriz (21 of 32 isolates), by contrast with the Tehran epidemiological landscape, where none of the identified strains was largely predominant. This might also result from the adoption of more effective infection control measures in the burn care centre compared with acute general hospitals.

In summary, this is the first report of the molecular epidemiology of A. baumannii from two Iranian cities using MLST. Our data thus provide early clues to the circulating clones in this country. Our study shows intraclonal diversity of the patterns of resistance determinants in IC I and II. The association between drug resistance and epidemicity on a reciprocal basis emphasizes the need for intensive control regimens and strict adherence to surveillance programmes to minimize the spread of multidrug-resistant A. baumannii.

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Transparency declarations

None to declare.

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