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Molecular Epidemiology and Characterization of Multiple-Drug Resistant (MDR) Clinical Isolates of *Acinetobacter baumannii*

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Abstract

Objectives—We aimed to identify the genetic relatedness of multiple-drug resistance (MDR) in *Acinetobacter baumannii* clinical isolates recovered from a hospital in Los Angeles.

Methods—Twenty one MDR *A. baumannii* isolates were collected and their antibiotic susceptibility were determined according to the CLSI guidelines. Genes coding for antibiotic resistance were identified by PCR and their identities were confirmed by DNA sequencing. Clonal relationships were studied by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST).

Results—MDR consistently correlated with the presence of oxacillinases, mostly in the form of plasmid-mediated OXA-23 enzyme which were detected in 12 (57.1%) isolates. GES-type carbapenemases were found in 20 (95.2%) strains, AAC in all 21 (100%) strains, PER in 7 (33.3%) strains and IS*Aba1* has been detected in 16 (76.2%) isolates. The association between IS*Aba1* and resistant genes confirms insertion elements as a source of β-lactamase production. Of the 21 clinical isolates, 5 were found to be related to sequence type-1 (ST1) and 16 to ST2 as analyzed by MLST. PFGE demonstrated that the majority of clinical isolates are highly related (>85%).

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Competing Interests:
The authors declare that there are no competing interests.

Authors' Contributions:
SS, AD, and ASI conceived and designed the experiments. SS, LV and MB performed the experiments. SS, LV and ASI analyzed the data. SS and ASI wrote the paper. AD, LV and MB revised the paper. All authors have approved the final article.

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Conclusions—This study supports a more complete understanding of genotyping of antibiotic resistance for better assessment of MDR strains transmission.

Keywords

A. baumannii; β-lactamase; MLST; PFGE

1. Background

Acinetobacter baumannii has emerged as a predominant cause of healthcare-associated infections (including those seen in wounded soldiers) both in the United States and world-wide.[1–10] *A. baumannii* infections include pneumonia (especially ventilator associated pneumonia), wound infections, urinary tract infections, septicemia and surgical site infections.[11–13] Risk factors for *A. baumannii* infections, especially in elderly individuals, include; patients having underlying diseases (e.g. diabetes), immune suppression, burns, trauma, invasive medical procedures, mechanical ventilation, catheters, previous antibiotic treatments and extended hospital stay.[14] Of great concern is the recent rise in the frequency of multiple drug resistant (MDR) and extremely drug resistant (XDR)- *A. baumannii* infections.[2,11,12] The percentage of *A. baumannii* infections caused by MDR strains (defined as resistance to 1 agent in at least three antimicrobial categories) and XDR strains (defined as resistance to all available antibiotics except colistin and tigecycline) has increased from <4% in 2000 to 60–70% in 2010.[14] Infections caused by MDR *A. baumannii* are associated with longer hospitalization, greater healthcare costs, greater morbidity, and >60% mortality for bloodstream infections as compared to drug-susceptible strains.[15–18] XDR infections are treatable only with second-line agents, such as tigecycline and colistin which are associated with clinical failure, development of resistance and nephrotoxicity.[16,19–28] Further, pandrug-resistant infections (PDR) are resistant to every FDA approved antibiotic, and are hence untreatable. Because of the difficulty in treating MDR, XDR and PDR *A. baumannii* infections, surveillance of *A. baumannii* isolates represent the cornerstone of prevention and control of these infections. In the current study, we aimed at characterizing the resistance mechanisms and determining the genetic relatedness of clinical isolates recovered from Harbor-UCLA Medical Center at Los Angeles County.

2. Methods

2.1. Bacterial Identifications

Twenty four clinical strains of *A. baumannii* obtained from in-patients Harbor-UCLA Medical Center (HUMC) of which 21 isolates were investigated and identified to the species level by using Vitek2 (BioMérieux Vitek Systems Inc., USA) and MicroScan (WalkAway System, Siemens, USA) systems utilizing biochemical methods.

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was determined for all HUMC strains of *A. baumannii* by automated broth microdilution method (Vitek2) (Vitek AMS; BioMérieux Vitek Systems Inc., USA) and the results were analyzed and interpreted using clinical breakpoints according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[29] The

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antibiotics tested were: Amikacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, ampicillin, cefazolin, cefepime, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefoxitin, cefpodoxime, cephalothin, ceftriazone, ciprofloxacin, gentamicin, imipenem, meropenem, levofloxacin, nitrofurantoin, norfloxacin, tetracycline, tobramycin, trimethoprim/ sulfamethoxazole, piperacillin/tazobactam, pipercillin, tigecycline, colistin and tigecycline. Extended spectrum β-lactamase (ESBL) production was confirmed by Vitek2 analyzer, Microscan and disk diffusion tests. Minimum inhibitory concentration (MICs) of quinolones, fluoroquinolones and β-lactams including carbapenems were determined using the E-test method (CLSI 2012).[29] Isolates that showed resistance to at least three classes of antibiotics were considered as MDR strains, whereas isolates showing resistance to all antibiotics except for colistin and tigecycline were considered as XDR strains.

2.3. Identification of Housekeeping Genes

Bacterial DNA was extracted using QIAquick PCR Purification Kit (Qiagen, USA). Primers used for polymerase chain reaction (PCR) amplifications of seven housekeeping genes in *A. baumannii* are listed in Table 1.

2.4. Antibiotic Resistance Genes by PCR and DNA Sequencing

The presence of resistant genes (Table 2) was investigated by PCR using GoTaq Green Master Mix (Promega, USA). PCR was conducted in a GeneAmp 9700 system (Perkin-Elmer, Illinois, USA) using the conditions specified for each primer as corresponding to the reference source. PCR was performed for metallo-β-lactamase and ESBL-encoding genes including *bla* SIM (Seoul imipenemase), *bla* VIM (Verona integron-encoded metallo- β-lactamases), *bla* VEB (Vietnamese extended-spectrum- β-lactamase) and *bla* IMP (Imipenemase), *bla* TEM-1 (Temoneira) and *bla* SHV (Sulfhydryl variable), *bla* CTX-M-like (Cefotaximase-Munchen),[30] *bla* NDM-1 (New-Delhi metallo- β-lactamase),[31] *qnr*A, *qnr*B and *qnr*S (Quinolone resistance genes),[32] *aac* (*N*-acetyltransferase),[33] *gyr*A (DNA gyrase subunit A) and *par*C (Topoisomerase IV subunit C),[34] *Amp*C (class C β-lactamases),[35] *bla* PER (Pseudomonas extended resistance),[36] *bla* GES (Guiana extended spectrum),[36] OXA-(oxacillinases)-encoding genes including *bla* OXA-51-like, *bla* OXA-58-like, *bla* OXA-48-like, *bla* OXA-23-like, *bla* OXA-24-like genes,[37,38] IS (insertion sequence),[39] and IS*Aba1*.[40] Amplified PCR products were purified with Qiagen purification kit (Qiagen, USA) according to the manufacturer's instructions and both strands were sequenced by automated AB13100 DNA sequencer (Applied BioSystems) system. The BLAST program of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov) was used to search and compare databases for similar nucleotide acid sequences.

2.5. Pulsed-Field Gel Electrophoresis

Pulsed-Field Gel Electrophoresis (PFGE) analysis was based on techniques described elsewhere.[41] PFGE of ApaI-digested genomic DNA (Promega, UK) from each HUMC strain was performed to detect the relationships among the clinical isolates of *A. baumannii*. After PFGE, the gels were stained with ethidium bromide and scanned. The analysis of gels was performed using BioNumerics software version 7.1 (Applied Maths, Ghent, Belgium).

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This software facilitates the development of the algorithms necessary for the comparison of profiles of isolates based on the Dice coefficient and the hierarchic unweighted pair arithmetic average algorithm. Cluster analysis and phylogenetic trees were subsequently analyzed with an optimization of 1.0% and a tolerance of 0.7%. Isolates were considered to belong to the same PFGE clone if their Dice similarity index was >85%.

2.6. Multi Locus Sequence Typing

Multi-locus sequence typing (MLST) was based on a sequence analysis of the internal fragments of seven housekeeping genes: *cpn60* (60-KDa chaperonin), *fusA* (elongation factor EF-G), *gltA* (citrate synthase), *pyrG* (CTP synthase), *recA* (homologous recombination factor), *rplB* (50S ribosomal protein L2), *rpoB* (RNA polymerase subunit B). The MLST scheme including amplification and sequencing primers, allele sequences and sequence types (STs) were available at Institute Pasteur's MLST web site (http://www.pasteur.fr/recherche/genopole/PF8/mlst/references_Abaumannii.html). The housekeeping genes for the MLST scheme were selected on the basis of their sequence availability in GenBank and prior studies of the phylogenetic relationships for the genus *Acinetobacter* and their presence in other MLST schemes available for other bacterial species. PCR primers were chosen from previous studies or were designed for amplification of the seven selected genes (Table 1).

All PCR amplifications were carried out using GoTaq Green Matser Mix (Promega, USA) under the following conditions: 35 cycles (denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 30 sec) proceeded by a 2 min denaturation at 94°C and followed by a 5 min extension at 72°C. PCR products were directly purified from the reaction mixture with the QIAquick PCR purification kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's recommendations. Sequencing of internal DNA fragments of 297 bp to 633 bp of the selected housekeeping genes was performed using ABI Prism 377 sequencer using the ABI Prism BigDye terminator cycle sequencing ready reaction kit V3.1 (PE Applied Biosystems, Foster City, CA) according to the manufacturer's recommendations. PCR primers were used for sequencing on both strands. Sequence data were aligned by CLUSTALW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

2.7. Plasmid Curing Experiments

Plasmid curing procedure was performed for five selected HUMC isolates (HUMC-1, HUMC-3, HUMC-4, HUMC-10, HUMC-19) of *A. baumannii* using 47°C as a growing temperature. The five isolates were selected to represent the presence or absence of resistance genes and IS*Aba1*. Plasmid curing using temperature as a curing agent were examined after 3 days incubation at 47C followed by antibiotic sensitivity testing.

3. Results

3.1. Antimicrobial Susceptibilities to *A. baumannii* Isolates

A total of 21 clinical samples were analyzed in 2011. The sources of the isolates included 9 samples of respiratory secretions (42.9%), 4 samples of sputum (19%), 2 samples from abdominal secretions (9.5%), 2 samples from wounds (9.5%) and one sample each from

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other sites (4.8%) including urine, bronchoalveolar lavage, foot and groin samples. Antibiotic sensitivity testing revealed that all the isolates were resistant to ceftriaxone, ceftazidime, cefotaxime, ciprofloxacin, cefepime, gentamicin, levofloxacin, tetracycline, ticarcillin/K clavulanate, whereas two isolates were found to be sensitive to meropenem, eight to imipenem, three to amikacin, four to ampicillin/sulbactam, one to each of trimethoprim/sulfamethoxazole tobramycin. All clinical isolates of *A. baumannii* were sensitive to colistin, and tigecycline with the exception of one and two strains, respectively. Susceptibility testing results of the studied clinical isolates are summarized in Table 3.

3.2. Characterization of Carbapenemases and other β-Lactamase Genes

We identified the presence of oxacillinases, mostly in the form of plasmid-mediated OXA-23 enzyme which were detected in 12 (57.1%) isolates as well as β-lactamase resistant genes in 7 isolates harboring PER (33.3%), 21 isolates harboring AAC (100%) and 20 isolates harboring GES (95.2%) -type enzymes (Table 4). MDR consistently correlated with the presence of oxacillinases, mostly in the form of plasmid-mediated OXA-51 and OXA-23 enzymes which were detected in 21 (100%) and 13 (61.9%) of the clinical isolates collected, respectively. None of the isolates harbored OXA-58, OXA-24 or OXA-48. ISAba1 was detected in 16 (76.2%) isolates. None of the clinical isolates harbored KPC, IMP, VIM, SIM, NDM, or QNR-type genes (Table 4).

3.3. Frequency of Insertion Sequences for Different Enzymes

The frequency of insertion sequences presence in all of the clinical isolates of *A. baumannii* harboring antibiotic resistance genes were found to be 7 out of 12 (58.3%) in OXA-23, 7 out of 7 (100%) in PER, 15 out of 20 (75%) in GES and 17 out of 21 (81%) in AAC (Table 4).

3.4. Molecular Genotyping of *A. baumannii* Clinical Isolates

Genotyping analysis of the 21 clinical isolates by PFGE revealed the circulation of different PFGE types. We found high clonal relationship among all the typed strains. All the isolates were MDR to at least three antimicrobial groups. PFGE analysis demonstrated that the majority of clinical isolates are highly related (85%). MLST studies have shown that 5 (23.8%) clinical isolates of *A. baumannii* were found to be related to sequence type-1 (ST1) and 16 (76.2%) belong to sequence type-2 (ST2) (Table 5). The results of PFGE and MLST are summarized in Figure 1, along with the information of the specimen original sources.

3.5. Plasmid Curing of *A. baumannii* Clinical Isolates

Our results have shown that the five clinical isolates cured for plasmids, did not lose their ability for resistance to antimicrobial agents. Plasmids curing using temperature as a curing agent failed even after 3 days incubation at 47°C (Table S1).

4. Discussion

Antimicrobial resistance in Enterobacteriaceae has emerged as a major clinical problem in recent years.[42,43] Drug resistance among this group of bacteria is mainly caused by the emergence and proliferation of extended-spectrum β-lactamases,[44] fluoroquinolone resistance,[45] and the dissemination of multiple drug-resistant (MDR) and carbapenem

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resistant strains.[46,47] Data from the National Healthcare Safety Network at the CDC showed high rates of carbapenem resistance among *A. baumannii* throughout USA with increased incidence in hospital-associated infections especially those of ventilated acquired pneumonia, central line associated bloodstream infections, catheter associated urinary tract infections and surgical site infections.[48] Understanding the fundamental mechanisms that underline *Acinetobacter* infections including the original sources of the infecting strains, resistance patterns, their clonality and geographical spread are critical for the development of appropriate infection control measures and more efficient treatment strategies.

We used two typing methods of PFGE and MLST to detect the molecular epidemiology of *A. baumannii* isolates from Harbor-UCLA Medical Center in Los Angeles County.[49,50] By using MLST we have shown that clinical isolates of *A. baumannii* belonged to two main clones; ST1 and ST2. The high clonal relationship in PFGE analysis between HUMC strains as reflected by 85% is in agreement with the MLST studies which showed more than 76.2% of the tested isolates belonged to ST2. It is prudent to mention that MLST is a high resolution molecular tool for discriminating between closely related bacterial species.[50] The first MLST scheme for *A. baumannii* was published by Bartual *et al.*[51] and by Diancourt *et al*.[52] at the Pasteur Institute (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html), which we have used in this study. Further, MLST approaches to genotype *A. baumannii* isolates, which are based on sequencing regions of housekeeping genes,[53] are reproducible and portable facilitating comparison among laboratories worldwide. While MLST is an expensive typing method due to the need for DNA sequencing, selective use of this technique can substantially enhance our understanding of molecular epidemiology across different hospitals and geographic locations. In contrast, although PFGE analysis is highly discriminatory, it is not suitable for inter-laboratory comparisons unless the procedures are meticulously standardized,[49] and the interpretation of the pulsed field results may be a challenge in non-outbreak situations.[50] However, genotyping by methods like PFGE allows investigation of clonal spread and can be used to identify the source of the original infection. Therefore, whenever possible the use of both methods for genotyping is advisable. Equally important, our previous work using PFGE analysis on 5 isolates of MDR *A. baumannii* demonstrated that all these isolates were genetically different from the drug susceptible *A. baumannii* ATCC 17978.[54]

Previous genome sequencing studies have shown that MDR *A. baumannii* strains causing infection are related to one another with extensive variation in gene content even among strains that were very closely related phylogenetically and epidemiologically.[55] Several mechanisms contribute to this diversity, including transfer of mobile genetic elements and mobilization of insertion sequences.[55] In addition, widespread genetic variation among clinical isolates from the same hospital and/or patient reinforces the need for molecular diagnostic testing and genomic analysis to determine resistance profiles, rather than to rely primarily on strain typing and antimicrobial resistance phenotypes for molecular epidemiological studies.[55]

The role of insertion sequences is important to understand the expression of carbapenemases in *A. baumannii* (e.g., IS*Aba1*). It has been reported that insertion sequences play a role in the expression of the carbapenem-hydrolyzing β-lactamases.[40] In this study, we have shown

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that IS*Aba1* was detected in almost 76.2% of the clinical isolates. We also showed that not all isolates harboring IS*Aba1* were resistant to carbapenems (Table 4). These findings are concordant with the fact that resistance to carbapenems is mainly caused by the OXA-type enzymes, including plasmid-encoded β-lactamases (OXA-23, OXA-40, and OXA-58).[38] Studies have shown that chromosomally encoded OXA β-lactamase (OXA-51-like) can confer resistance to carbapenems in *A. baumannii* when the genetic environment around the gene promoted its expression.[38,56] Our findings are in agreement with other studies in which we show that almost all *A. baumannii* strains possess chromosomally encoded OXA β-lactamase (OXA-51 like). The high frequency of insertion sequences for different types of enzymes, oxacillinases and carbapenemases series in *A. baumannii* could be due to differences in antibiotic treatment given to those patients. Our plasmid curing studies have shown that clinical strains did not lose their ability for resistance to antimicrobial agents, indicating a high stability of acquired plasmids.

The two clonal lineages of MDR *A. baumannii* identified in our study have been found in other States as well.[46] In the United States, an outbreak of MDR *A. baumannii* in Houston was caused by clonal complex 92 (an ST-2 type).[57] In addition, a survey of bacterial isolates collected from 52 U.S. hospitals over 6 years also showed the preponderance of the same clone.[58] Other studies has revealed good correlation between antibiotic susceptibility profiles and genetic fingerprints from clinical *A. baumannii* isolates from nosocomial outbreaks and the mechanisms of antibiotic resistance.[59] Molecular epidemiology of clinical isolates of *A. baumannii* identified in New York, Pennsylvania, Florida, Missouri, Nevada, and California revealed the predominance of CC92 among carbapenem non-susceptible isolates in US hospitals, suggesting that they constitute part of the global epidemic driven by this clonal complex belonging to EUII.[46] Worldwide, it is not surprising to detect common STs. For examples, ST-1 and ST-2 have been previously identified in the Middle East including Saudi Arabia, Lebanon and Yemen.[60–62] Moreover, ST-1 and ST-2 are known as endemic strains in European countries including Spain, Italy, France and Greece,[63–66] Asia including Japan and Taiwan,[67,68] and even the Scandinavian countries like in Denmark.[69]

There is no doubt that early pathogen identification, followed by the right antibiotic treatment may reduce the prevalence of antibiotic resistance in *A. baumannii.* Therefore, future studies will focus on characterizing the composite transposable elements in detail. Though, the clinical isolates collected in this study may not represent the overall epidemiology of MDR *A. baumannii* since it all originated from one hospital in Los Angeles County, we plan to further research the epidemiological analyses of this organism beside other MDR and XDR organisms in other hospitals for continuous surveillance of these strains in the United States.

5. Conclusion

Two distinct clones of MDR or XDR *A. baumannii* were identified at Harbor UCLA Medical Center in Los Angeles County. The epidemiological data obtained suggested that the increase in the number of *A. baumannii* infections in that hospital was caused by these two clones. MLST studies maybe more accurate in distinguishing between *A. baumannii* isolates than PFGE typing. This study supports a more complete understanding of

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genotyping of antibiotic resistance for better assessment of MDR and/or XDR strains transmission. Continuous surveillance is needed for monitoring the spread of these strains equipped with multiple drug resistance mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Oxacillinases correlated with 90% of MDR *A. baumannii* from a single hospital
- GES-type carbapenemases and ISAba1 were also detected.
- Insertion elements are likely behind resistance via β-lactamase production
- Majority of the strains belonged to sequence type-2
- Genotyping of resistance aids in understanding of MDR *A. baumannii* transmission

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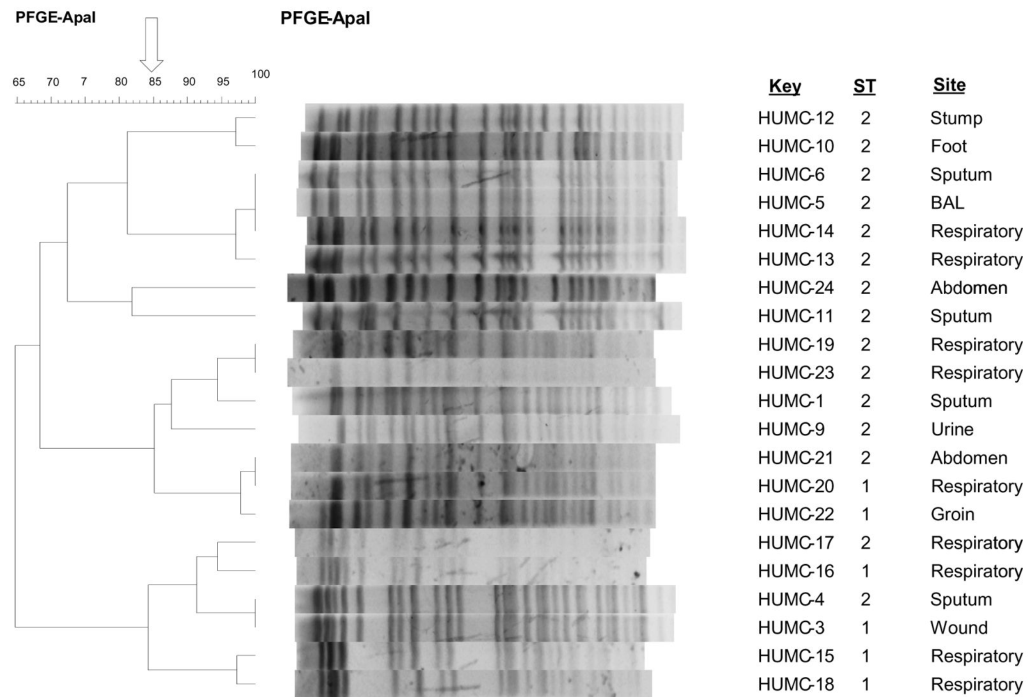

Figure 1.

Dendrogram representing PFGE profiles, MLST results and site of infection of HUMC clinical isolates.

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Table 1

Gene primers used for housekeeping genes detection by PCR in genes in clinical *A. baumannii* isolates in MLST studies.

Gene	Primer Sequence	Amplicon Size
cpn60-F ***cpn60-R***	ACTGTACTTGCTCAAGC TTCAGCGATGATAAGAAGTGG	405 bp
fusA-F ***fusA-R***	ATCGGTATTTCTGCKCACATYGAT CCAACATACKYTGWACACCTTTGTT	633 bp
gltA-F ***gltA-R***	AATTTACAGTGGCACATTAGGTCCC GCAGAGATACCAGCAGAGATACACG	483 bp
pyrG-F ***pyrG-R***	GGTGTTGTTTCATCACTAGGWAAAGG ATAAATGGTAAAGAYTCGATRTCACCMA	297 bp
recA-F ***recA-R***	CCTGAATCTTCYGGTAAAAC GTTTCTGGGCTGCCAAACATTAC	372 bp
rplB-F ***rplB-R***	GTAGAGCGTATTGAATACGATCCTAACC CACCACCACCRTGYGGGTGATC	330 bp
rpoB-F ***rpoB-R***	GGTCCTGGTGGTTTAACACG CGAATAACGATACGAGAAGCA	456 bp

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Table 2

Gene primers used for PCR amplification of antibiotic resistance genes in clinical *A. baumannii* isolates.

Gene	Amplicon Size	Tm °C	Primer Sequence
OXA-58	599bp	52	AAGTATTGGGGCTTGTGCTG (Forward) CCCCTCTGCGCTCTACATAC (Reverse)
OXA-51	353bp	52	TAATGCTTTGATCGGCCTTG (Forward) TGGATTGCACTTCATCTTGG (Reverse)
OXA-24	246bp	52	GGTTAGTTGGCCCCCTTAAA (Forward) AGTTGAGCGAAAAGGGGATT(Reverse)
OXA-23	501bp	52	GATCGGATTGGAGAACCAGA (Forward) ATTCTGACCGCATTTCCAT(Reverse)
OXA-48	438bp	62	GCGTGGTTAAGGATGAACAC (Forward) CATCAAGTTCAACCCAACCG (Reverse)
CTXM	550bp	60	CGCTTTGCGATGTGCAG (Forward) ACCGCGATATCGTTGGT (Reverse)
CTXM2	896bp	55	CGGAATTCATGATGACTCAGAGCATTCG (Forward) GCTCTAGATTATTGCATCAGAAACCGTG (Reverse)
PER	900bp	43	ATGAATGTCATTATAAAAGC (Forward) AATTTGGGCTTAGGGCAGAA (Reverse)
VEB	600bp	55	CGACTTCCATTTCCCGATGC (Forward) GGACTCTGCAACAAATACGC (Reverse)
QnrA	580bp	54	AGAGGATTTCTCACGCCAGG (Forward) TGCCAGGCACAGATCTTGAC (Reverse)
QnrS	428bp	54	GCAAGTTCATTGAACAGGGT (Forward) TCTAAACCGTCGAGTTCGGCG (Reverse)
QnrB	264bp	54	GGMATHGAAATTCGCCACTG (Forward) TTTGCYGYYCGCCAGTCGAA (Reverse)
TEM	850bp	42	GAGTATTCAACATTTCCGTGTC (Forward) TAATCAGTGAGGCACCTATCTC (Reverse)
GES	846bp	55	ATGCGCTTCATTCACGCAC (Forward) CTATTTGTCCGTGCTCAGGA (Reverse)
SHV	861bp	55	ATGCGTTATWTTCGCCTGTGT (Forward) TTAGCGTTGCCAGTGCTCG (Reverse)
CTX	554bp	60	TCTTCCAGAATAAGGAATCCC (Forward) CCGTTTCCGCTATTACAAAC (Reverse)
GyrA6	620bp	56	CGACCTTGCGAGAGAAAT (Forward) GTTCCATCAGCCCTTCAA (Reverse)
ParCF43	964bp	53	AGCGCCTTGCGTACATGAAT (Forward) GTGGTAGCGAAGAGGTGGTT (Reverse)
AAC	482bp	55	TTGCGATGCTCTATGAGTGGCTA (Forward) CTCGAATGCCTGGCGTGTTT (Reverse)
SIM	570bp	65	TACAAGGGATTCGGCATCG (Forward) TAATGGCCTGTTCCCATGTG (Reverse)
IMP	232bp	60	GGAATAGAGTGGCTTAAYTC (Forward) TCGGTTTAAYAAAACAACCACC (Reverse)
VIM	390bp	62	GATGGTGTTTGGTCGCATA (Forward) CGAATGCGCAGCACCAG (Reverse)
NDM	621bp	65	GGTTTGGCGATCTGGTTTTC (Forward) CGGAATGGCTCATCACGATC (Reverse)
KPC	798bp	58	CGTCTAGTTCTGCTGTCTTG (Forward) CTTGTCATCCTTGTTAGGCG (Reverse)

Gene	Amplicon Size	Tm °C	Primer Sequence
IS	615bp	56	GTGCCCAAGGGGAGTGTATG (Forward) ACYTTACTGGTRCTGCACAT (Reverse)
ISAbaI	389bp	57	ATGCAGCGCTTCTTTGCAGG (Forward) AATGATTGGTGACAATGAAG (Reverse)

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Table 3

Susceptibility profiles of HUMC strains. Isolates were designated susceptible (S), intermediate (I), or resistant (R) according to CLSI antibiotic breakpoint guidelines. Minimum inhibitory concentrations (MIC) were shown in brackets.

Antibiotic/Isolate	A/S	AK	CAZ	CP	CPE	GM	IMP	LVX	MER	T/S	TE	TIM	TO	CST	TGC
HUMC-1	I (16/8)	S (16)	R (>32)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-3	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	R (4)
HUMC-4	I (16/8)	S (16)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	S (4)	S (2)	I (2)
HUMC-5	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-6	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-9	S (8/4)	S (16)	R (>16)	R (>2)	R (>16)	R (>8)	I (8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	I (2)
HUMC-10	S (8/4)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	S (4)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-11	R (>16/8)	R (>32)	R (>16)	R (>2)	I (16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-12	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	S (4)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-13	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-14	R (>16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-15	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	R (4)
HUMC-16	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	S (2/38)	I (8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-17	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-18	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	I (2)
HUMC-19	S (8/4)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-20	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-21	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	R (>8)	R (>64)	R (>8)	R (>2)	S (1)
HUMC-22	I (16/8)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	I (8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-23	S (8/4)	R (>32)	R (>16)	R (>2)	R (>16)	R (>8)	R (>8)	R (>4)	R (>8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)
HUMC-24	R (>16/8)	R (>32)	R (>16)	R (>2)	I (16)	R (>8)	S (4)	R (>4)	I (8)	R (>2/38)	R (>8)	R (>64)	R (>8)	S (2)	S (1)

Abbreviations: A/S, Ampicillin/Sulbactam; AK, Amikacin; CAZ, Ceftazidime; CP, Ciprofloxacin; CPE, Cefepime; GM, Gentamicin; IMP, Imipenem; LVX, Levofloxacin; MER, Meropenem; T/S, Trimethoprim/Sulfamethoxazole; TE, Tetracycline; TIM, Ticarcillin/K Clavulanate; TO, Tobramycin; CST, Colistin; TGC, Tigecycline.

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Table 4

Antibiotic resistance genes results detected by PCR in HUMC clinical strains. Abbreviations: (−) denotes negative, (+) denotes positive PCR reaction.

Gene/Isolate	OXA-51	OXA-23	PER	GES	AAC	ISAba1
HUMC-1	+	+	−	+	+	+
HUMC-3	+	+	−	+	+	−
HUMC-4	+	−	+	+	+	+
HUMC-5	+	−	+	+	+	+
HUMC-6	+	−	+	+	+	+
HUMC-9	+	+	−	+	+	+
HUMC-10	+	−	−	+	+	+
HUMC-11	+	−	+	+	+	+
HUMC-12	+	−	−	+	+	+
HUMC-13	+	−	+	+	+	+
HUMC-14	+	−	+	+	+	+
HUMC-15	+	+	−	+	+	−
HUMC-16	+	+	−	+	+	+
HUMC-17	+	+	−	+	+	+
HUMC-18	+	+	−	+	+	−
HUMC-19	+	+	−	−	+	+
HUMC-20	+	+	−	+	+	+
HUMC-21	+	+	−	+	+	−
HUMC-22	+	+	−	+	+	−
HUMC-23	+	+	−	+	+	+
HUMC-24	+	−	+	+	+	+

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Table 5

Allele and sequence number results of HUMC clinical isolates as analyzed by MLST.

HUMC Strains	Allele Number							ST
	Cpn60	*fusA*	*gltA*	*pyrG*	*recA*	*rplB*	*rpoB*	
HUMC-1	2	2	2	2	2	2	2	2
HUMC-3	1	1	1	1	5	1	1	1
HUMC-4	2	2	2	2	2	2	2	2
HUMC-5	2	2	2	2	2	2	2	2
HUMC-6	2	2	2	2	2	2	2	2
HUMC-9	2	2	2	2	2	2	2	2
HUMC-10	2	2	2	2	2	2	2	2
HUMC-11	2	2	2	2	2	2	2	2
HUMC-12	2	2	2	2	2	2	2	2
HUMC-13	2	2	2	2	2	2	2	2
HUMC-14	2	2	2	2	2	2	2	2
HUMC-15	1	1	1	1	5	1	1	1
HUMC-16	1	1	1	1	5	1	1	1
HUMC-17	2	2	2	2	2	2	2	2
HUMC-18	1	1	1	1	5	1	1	1
HUMC-19	2	2	2	2	2	2	2	2
HUMC-20	2	2	2	2	2	2	2	2
HUMC-21	2	2	2	2	2	2	2	2
HUMC-22	1	1	1	1	5	1	1	1
HUMC-23	2	2	2	2	2	2	2	2
HUMC-24	2	2	2	2	2	2	2	2