Genetic Characterization of Extensive Drug Resistant Acinetobacter Baumannii: an Appalling Impediment

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Abstract

Introduction: Acinetobacter baumannii infections are a growing public-health concern. The bacterium's potentiality to acquire resistance to a number of commonly used antibiotics has turned it into a formidable pathogen.

Aims: Molecular characterization of extensive drug resistant (XDR) typing of A. baumannii clinical isolates by polymerase chain reaction.

Materials and methods: Thirty XDR A. baumannii were investigated for the presence of genes encoding carbapenemase resistance, biofilm capacity, autoinducer synthase, virulence and surface motility by polymerase chain reaction (PCR). Later, the isolates were typed by plasmid-based replicon (Rep) (PBRT) and trilocus sequence typing.

Results: All 30 XDR A. baumannii strains displayed genes related to surface motility, autoinducer synthase, virulence determinant, biofilm related genes except PER, and bap, the frequency of which was 83.3% and 76.6%, respectively. Analysis of rep genes showed highest frequency of rep6 and rep2 genes, with frequency of 75% and 65%, respectively. All XDR A. baumannii strains belonged to SG I (European clone II) group.

Conclusions: Our results show the extraordinary plasticity of XDR A. baumannii and suggest that the strains have gained endemicity in our hospital, which could be a great concern in the near future.

Keywords

Acinetobacter baumannii, autoinducer synthase, biofilm, carbapenem-hydrolyzing class D β-lactamases, extensive drug-resistance, replicase typing, trilocus sequence typing, virulence
INTRODUCTION

Acinetobacter baumannii, famed as an "old friend-new enemy", has emerged as a successful pathogen causing a broad array of clinical infections in hospital and community acquired settings. The organism possesses intrinsic and acquired resistance to a number of commonly used antibiotics and is thus gradually bequeathing intra and inter-hospital setting. Nevertheless, antibiotic resistance rates can vary according to the country, the individual hospital, and may even depend on biological, epidemiological or methodical factors. As the organism has gained hold of the antibiotic resistance, it is not uncommon to find multidrug-resistant (MDR, resistance to at least three classes of antimicrobials), extensively drug-resistant (XDR, MDR plus resistance to carbapenems), and pan-drug-resistant (PDR, XDR plus resistance to polymyxins) nosocomial isolates that are hard to treat with the currently available drugs. Amongst all these, according to World Health Organization (WHO) carbapenem-resistant A. baumannii (CRAB) strains have emerged as one of the most concerning antibiotic-resistant pathogens among other gram-negative bacteria.

Carbapenem-hydrolyzing class D β-lactamases (CHDLs) are determinants of carbapenem resistance in A. baumannii. Four major plasmid encoded CHDLs genes including blaoXA-24/40, blaoXA-23', blaoXA-58, and blaoXA-51-like, have been documented globally. Amongst them, strains carrying blaoXA-58 stand out as the most common type from Europe, Argentina, Australia, the United States and many Asian countries.

One of the major factors contributing to drug resistance in A. baumannii associated infections is its biofilm development capacity. Quorum sensing (QS) (autoinducer-receptor mechanism) plays a role in biofilm formation in Acinetobacter baumannii associated infections, though its role in the regulation of other virulence factors is yet to be established. Several published studies have explored the relationship between biofilm and antibiotic resistance in A. baumannii. In this regard, A. baumannii is among the leading nosocomial pathogens with a capacity to colonize venous catheters (CVCs) and cause lower respiratory tract infections (due to contaminated ventilators). Bacteria in the biofilm structure are included in a matrix that increases drug resistance and causes chronic and persistent diseases that are difficult to treat. Research conducted on biofilm-associated operon in A. baumannii suggested CsaA/BABCDDE-mediated pili formation, which plays a role in the initial steps of biofilm by allowing bacterial cells to adhere to abiotic surfaces resulting in the initiation of microcolony formation that precedes the full development of biofilm structures. CsaE codes for the tip adhesion and its inactivation result in the abolition of pili production as well as biofilm formation. The expression of this operon has been found to be regulated by a two-component system comprising sensor kinase encoded by bfmS and a response regulator bfmR. BfmSR controls the production of capsular exopolysaccharides as well as pilus assembly, and consequently, cell attachment and biofilm formation. A. baumannii also contains pgaABCD locus that encodes a protein which synthesizes cell-associated poly-β-(1-6)-N-acetyl glucosamine (PNAG). In a variety of gram-negative bacteria, it has been demonstrated that biofilm development may depend on N-acetyl-homoserine lactone (AHL) signalling molecules. Mass spectrometry has identified AHL signals directed by protein Abal. The abal gene is activated in a positive-feedback loop by an Abal-dependent AHL signal(s). In addition, a homolog of a staphylococcal biofilm-associated protein (Bap) has been characterized in A. baumannii, where it appears to act as an extracellular adhesin and play a key role in biofilm production in A. baumannii. blaPER-1 and Bap, in addition to being involved in biofilm formation, are also involved in the bacterial attachment to human epithelial cells and abiotic surfaces. The formation of pellicles, a specific form of biofilm, occurs at the air-liquid interface and is distinct from submerged biofilms. A correlation between surface associated motility and pellicle biofilm formation has been described for A. baumannii.

The organism encodes a diverse range of secretion systems. The type I secretion system (T1SS) is a tripartite system, delivering proteins from the cytosol to the extracellular environment. Interestingly, the activity of the T1SS was shown to have a direct impact on the type VI secretion system (T6SS), suggestive of cross talk between these systems. Among various virulence factors possessed by A. baumannii, outer membrane protein A (OmpA, previously Omp38) is the most abundant A. baumannii OMP and one of the most well-characterized virulence factors.

Despite the progress in the study of antibiotic resistance mechanisms in A. baumannii, a more reconcile work is knowledge about the genetic factors that have driven the recent evolution of A. baumannii toward multidrug resistance. Sequence analysis of plasmid replications corresponding to A. baumannii clinical strains has revealed many differences with those from other bacterial species, strongly suggesting that A. baumannii contains its own plasmid types. Research on the genome content of A. baumannii species reveals the specificity of its plasmids and thus, plasmid typing of A. baumannii according to their replicase (Rep) proteins was suggested as an effective tool. Plasmid-based repiclon (Rep) (PBRT) typing scheme furnished that there are 19 homology groups (GR1–GR19) based on their nucleotide sequence similarities. PBRT method is particularly useful when investigating specific features such as an antibiotic resistance gene. Intriguingly, it will be a very useful method to detect and study these plasmids and to further understand the evolution of resistance.

A multilocus sequence-typing scheme or the “trilocus sequence-based typing” (3LST), based on housekeeping genes, for A. baumannii examined the sequence variation within three genes likely to be under selective pressure. This approach, which has the potential for greater discrimination, comprises of three genes: ompA, cseA and blaoXA-51-like.
Outer-membrane protein A (encoded by ompA) is a porin found to induce apoptosis of epithelial cells. The csuE gene codes for part of a pilus assembly system and is essential for biofilm formation. The blaOXA-51-like gene codes for the intrinsic carbapenemase found in A. baumannii. The 3LST-based multiplex-PCR assay’s purpose was to rapidly assign isolates to profile groups (G), consisting of G1, corresponding to ICI; G2, to ICI; and G3, to ICIII. Subsequently, several studies using this PCR-based typing technique reported the identification of new amplicon combinations, in addition to those expected for ICI-III clones.

**AIM**

The present study determined I) the correlation between the ability of A. baumannii for biofilm formation and distribution of biofilm related genes, and II) carbapenem resistance genotypes in the XDR A. baumannii clinical isolates. Furthermore, we applied PBRT and 3LST schemes to type the isolates according to their replicase and housekeeping genes, respectively.

**MATERIALS AND METHODS**

**Bacterial isolates**

We performed this study on 30 XDR A. baumannii strains isolated from clinical specimens obtained from various patients admitted in Sina Hospital, a University based Teaching and Research Center (Tabriz, Iran) as a routine bacteriological procedure. The A. baumannii isolates were initially identified by conventional phenotypic methods and later confirmed genotypically (blaOXA-51 and rpo genes). Acinetobacter baumannii ATCC19606 was used as a standard strain.

**Antimicrobial susceptibility testing**

The antibiotic susceptibility of A. baumannii isolates was based on the disk diffusion and agar dilution (minimum inhibitory concentration, MIC) methods. The disk diffusion was done by inoculating bacterial culture (turbidity matched equivalent to 0.5 McFarland standard) onto Mueller Hinton agar plate, according to Clinical Laboratory Standard Institute (CLSI) guidelines. Antibiotics used to assess the susceptibility of A. baumannii isolates were: cefazidime (30 μg), cefepime (30 μg), sulfamethoxazole/trimethoprim (1.25/23.75 μg), amikacin (30 μg), gentamicin (10 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), ampicillin-sulbactam (10 μg/10 μg), imipenem (10 μg), and meropenem (10 μg) (Mast Group Co, UK). The interpretative zones were measured and interpreted as per CLSI recommendations. The MIC of colistin and tigecycline (Sigma-Aldrich, St Louis, MO, USA) was determined by agar dilution by preparing serial dilutions (from 0.25 to 256 μg/mL) of above mentioned antibiotics as per CLSI guidelines. Extensive-drug resistance (XDR) was defined in this analysis as resistance to all following drug classes except colistin and tigecycline. Extended-spectrum cephalosporins (ceftazidime and cefepime), aminoglycosides (amikacin and gentamicin), folate pathway inhibitors (sulfamethoxazole/trimethoprim), quinolones (ciprofloxacin) and carbapenems (imipenem and meropenem) were used for biofilm formation and dispersal, though not consistent, antimicrobial activity against MDR (including carbapenem-resistant) A. baumannii isolates. A research study conducted concluded that a breakpoint zone diameter of ≥16/≤12 mm to define susceptibility/resistance, respectively, instead of those proposed by the U.S. Food and Drug Administration (FDA) for Enterobacteraceae organisms (≥19/≤14 mm, respectively), reduces the intermethod minor errors to an acceptable level (only 9.7% instead of 23.3%, with the FDA breakpoints proposed).

**Detection of blaOXA genes**

The bacterial genomic DNA was extracted from overnight cultures of A. baumannii isolates using a DNA purification kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Multiplex-PCR was performed to investigate the presence of carbapenemase genes: blaOXA-143, blaOXA-23, blaOXA-24, blaOXA-51, blaOXA-58, using primers and amplification conditions as described previously. The primers used are listed in the Table 1.

**Quantitative biofilm formation assay**

Clinical XDR A. baumannii isolates were analyzed for their ability to produce biofilm using microtiter plate method based on the crystal violet staining method. Briefly, isolates were initially cultured in the Tryptic Soy Broth (TSB) medium comprising 1% glucose. After incubation for 24 hours at 37°C, 20 μl of fresh bacteria containing medium was transferred to the 96-well polystyrene microtiter well containing 180 μl of fresh TSB medium. After incubation at 37°C for 24 hours, the medium was discarded and the adherent cells washed twice with the phosphate-buffered saline (PBS) (pH7.4) followed by addition of 250 μl (99%) methanol and then 200 μl (0.1%) Crystal Violet. The stain was eluted from the adherent cells using 160 μl acetic acid (33%). Wells containing no bacteria were used as controls. Absorbance (optical density) of the eluted solvent was measured for the clinical isolates (ODI) and negative control (ODC) at 570 nm using an UV visible spectrophotometer (Epoch, Biotek). The assay was repeated at least three
times using fresh samples each time. The results were interpreted as follows: if \( ODi < ODc \), the bacteria were non-adherent; if \( ODc < ODi - 2 \times ODc \), the bacteria were weakly adherent; if \( 2 \times ODc < ODi < 4 \times ODc \), the bacteria were moderately adherent; and if \( 4 \times ODc < ODi \), the bacteria were strongly adherent.40

Detection of biofilm (bap, PER, bfmSR, csuE, pgaA, and pgaD), motility (type I fimbriae, PilT), autoinducer synthase (abal) and virulence related genes (omp)

The bacterial genomic DNA was extracted as stated above according to manufacturer’s protocol. Amplification of biofilm associated, autoinducer synthase, virulence and surface motility-related genes was performed using specific primers (listed in Table 1) and PCR conditions in XDR Acinetobacter baumannii isolates.41,42

A. baumannii sequence-based typing method

Two sets of multiplex-PCR (SG1 and SG2) were performed with primers targeting ompA, csuE and blaOXA-51-like specific alleles.30 PCR program included an initial denaturation at 94°C for 3 min followed by 30 cycles of 45 s denaturation at 94°C, 45 s primer annealing at 57°C, 1 min extension at 72°C; and a final elongation step of 72°C for 5 min.30

A. baumannii PCR-based replicon typing (AB-PBRT) method

The bacterial genomic DNA was extracted from overnight cultures of A. baumannii isolates in LB broth using a DNA purification kit (Qiagen, Hilden, Germany) according to manufacturer’s protocol. The primers used for AB-PBRT

| Trait | Gene | Sequence | Size | Amplification temperature | Ref Reference |
|-------|------|----------|------|---------------------------|---------------|
| Virulence | ompA-F | AGCATAAAAGAAGCTACACCTGC | 154bp | 60.5 | (41) |
| Virulence | ompA-R | AAAGTGGCCCAAAGAAAACCTTGA | | | |
| Pili and motility | fim1-F | GACATTGGTAGCTGCACCAAG | 384bp | 61 | |
| Pili and motility | fim1-R | GATGTTGCTGTCGTCAACC | | | |
| Pili and motility | PilT-F | AGTGTAACAAACCAACAGTGA | | | |
| Pili and motility | PilT-R | TCGGAGTAATCAACTAGCCTTG | 150bp | 58.5 | (41) |
| Biofilm, quorum sensing, Autoinducer synthase | bfmR-F | GGCATCGCTTCCATCTTGGATGTCA | 348bp | 60 | |
| Biofilm, quorum sensing, Autoinducer synthase | bfmR-R | GATAAAAATACGGCCAGTGTTTG | | | |
| Biofilm, quorum sensing, Autoinducer synthase | bms-F | CAGTTTGGTTGGTTGGTGACAGA | 474bp | 60 | |
| Biofilm, quorum sensing, Autoinducer synthase | bms-R | TTGCTGTTTACACACCAAACTGACCT | 564bp | 58 | |
| Biofilm, quorum sensing, Autoinducer synthase | csuE-F | TTGCGTTTACACACCAAACTGACCT | 150bp | 58.5 | (41) |
| Biofilm, quorum sensing, Autoinducer synthase | csuE-R | TCGGAGTAATCAACTAGCCTTG | | | |
| Biofilm, quorum sensing, Autoinducer synthase | pgaA-F | GCAAATAATCCTTCCATGCTT | 670bp | 57.5 | |
| Biofilm, quorum sensing, Autoinducer synthase | pgaA-R | GTTTTGGTAGCTGTTTGGTGACAGA | | | |
| Biofilm, quorum sensing, Autoinducer synthase | bap-F | GTGACAAACATATGTCGGCGAATT | 934bp | 61 | |
| Biofilm, quorum sensing, Autoinducer synthase | bap-R | CTTGATTCACTCCTTGGACCAGC | | | |
| Biofilm, quorum sensing, Autoinducer synthase | abal-F | CCACACACCAACCTATTCTACCGG | 121bp | 58 | |
| Biofilm, quorum sensing, Autoinducer synthase | abal-R | GCCGTTTGTGAAAATCTACGG | | | |
| Antibiotic Resistance | blaPER-F | TTGATCGACCCCTGAAATGGTA | 145bp | 60 | (42) |
| Antibiotic Resistance | blaPER-R | CACACATAGTCATAAATGGGA | | | |
| Antibiotic Resistance | OXA-23-F | AGCATAAAAGAAGCTACACCTGC | 501bp | 51 | (72) |
| Antibiotic Resistance | OXA-23-R | AAAGTGGCCCAAAGAAAACCTTGA | | | |
| Antibiotic Resistance | OXA-40-F | ATGTTGCGACAAACAGA | 246bp | | |
| Antibiotic Resistance | OXA-40-R | AGTTTGACGACAAACAGA | | | |
| Antibiotic Resistance | OXA-51-F | TAAAGGCCATCATTCCGCTTG | 353bp | 52 | (39) |
| Antibiotic Resistance | OXA-51-R | TGGATGGCATTCTATCATTGG | | | |
| Antibiotic Resistance | OXA-58-F | AGAATGGCGGGTTCGTTCGT | 599bp | | |
| Antibiotic Resistance | OXA-58-R | GCCCCTTGGCGCTACATTAC | | | |
| Antibiotic Resistance | OXA-143-F | TTGATCGACCCCTGAAATGGTA | 728bp | | |
are listed in Table 2. The PCR amplifications for 19 rep genes were grouped as 11 simplex PCR or as duplex PCR (Table 2). PCR amplifications were performed as follows: 1 cycle of denaturation at 94°C for 7 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing (mentioned in Table 2) and elongation at 72°C for 1.5 min. The amplification was ended with an extension program at 72°C for 5 min.

Statistical analysis

As all thirty A. baumannii isolates were positive for almost all the genes studied for biofilm, autoinducer synthase, virulence and surface motility-related, thus only the frequencies were calculated as percentages. The association between the genes involved in biofilm formation and the amount of biofilm mass with antibiotic resistant phenoty-

| Replicase groups | Gene  | Sequence                      | Size  | Amplification temperature | Ref |
|------------------|-------|-------------------------------|-------|---------------------------|-----|
| Group 1          | gr1-F | CATAGAAATACAGCTATAAAG         | 330bp | 52                        |     |
|                  | gr1-R | TTCTTTCTGCTCTACAAAAAT         |       |                           |     |
| Group 2          | gr2-F | AGTAGAACAAAGTTTATTTATGGCC     | 851bp | 52                        |     |
|                  | gr2-R | CACCTTTTTTTAGGTATGGTATAG      |       |                           |     |
|                  | gr3-F | TAATTATGCGATTATAACCTTTG       | 505bp |                           |     |
|                  | gr3-R | GTACGAGTACACTTTTGGT          |       |                           |     |
| Group 3          | gr4-F | GTCCATGCTGAGACTATGT          | 508bp |                           |     |
|                  | gr4-R | TAGCTCCTTTTTATGTTGC          |       |                           |     |
|                  | gr9-F | GCAAGTTACATATAGCCT           | 191bp |                           |     |
|                  | gr9-R | AAAATAAACGCTCTGATGC          |       |                           |     |
| Group 4          | gr5-F | AGAACAGTCGTTGGACTAAT         | 220bp |                           |     |
|                  | gr5-R | GACCGTGGGCTACGTGTAAC         |       |                           |     |
|                  | gr11-F| GCTTATCTCCTACTCTTACTTTT      | 852bp |                           |     |
|                  | gr11-R| GTTTCCTCTTTACACTTTT          |       |                           |     |
| Group 5          | gr6-F | AGGACAGTACGTTGGACTAAT        | 662bp |                           |     |
|                  | gr6-R | AAGCAATGAAACAGCCTAAAT        |       |                           |     |
|                  | gr16-F| CTCGAGTACGTTGGACTAAT         | 233bp |                           |     |
|                  | gr16-R| GCCATTCTGAGATCAACAAAC        |       |                           |     |
| Group 6          | gr7-F | GAAACAGTATGTTGTAAGA          | 885bp | (27)                      |     |
|                  | gr7-R | TCCTCTAAATTTTTAGGGCTCT       |       |                           |     |
|                  | gr18-F| TGGGGTATCATACAAATACA         | 676bp |                           |     |
|                  | gr18-R| TGAACATGGGAACCTCTCAAT        |       |                           |     |
| Group 7          | gr8-F | AATTATCGTTAAAGGATAATAGC      | 233bp |                           |     |
|                  | gr8-R | GACATGACGATTCAAAATACAAG      |       |                           |     |
|                  | gr14-F| TAAAATGGGTCGGTTAATT          | 622bp |                           |     |
|                  | gr14-R| GCCATCTTCTCAAAAACCTTG        |       |                           |     |
| Group 8          | gr10-F| TTTTCACTAGTACCAACTA          | 371bp |                           |     |
|                  | gr10-R| AACACGGTGTGGTGGCAGTC         |       |                           |     |
|                  | gr13-F| CAAGATCAGAATCAGA             | 780bp |                           |     |
|                  | gr13-R| CTGTTTATAATTGGGCTGT          |       |                           |     |
| Group 9          | gr12-F| TCATTGGAATTCGTTTTCAAAACC     | 165bp |                           |     |
|                  | gr12-R| ATTTCAGCCCTACCTATTTGTC       |       |                           |     |
|                  | gr15-F| GGAATAAAAAATGATGAGTC         | 876bp |                           |     |
|                  | gr15-R| AIAAGTTTGGTGGGTATATTG        |       |                           |     |
| Group 10         | gr17-F| AATAAACCTTAAATACCTTGTA       | 380bp |                           |     |
|                  | gr17-R| GCAAATGTTGACCTCTAATA         |       |                           |     |
|                  | gr19-F| ACAGGAGTACAAACATGGCTCA       | 815bp |                           |     |
|                  | gr19-R| AGCTGACATTTCCAGCATT          |       |                           |     |
types of *A. baumannii* was evaluated using software IBM SPSS Statistics version 25.0 (IBM Corp., USA). The analysis was performed with a confidence level of 95%. *P* values < 0.05 were considered statistically significant.

**RESULTS**

**Bacterial isolates**

Of 30 XDR *A. baumannii* clinical isolates enrolled in the study, 16 (53.3%) isolates were obtained from male patients and 14 (46.6%) from females. *A. baumannii* isolates were recovered from tracheal aspirate and wounds (each *n*=9; 30%), blood (*n*=7; 23.3%), IV catheter (*n*=1; 3.3%), and urine (*n*=4; 13.3%).

**Antibiotic susceptibility testing**

On disk diffusion assay, all isolates showed no zone of inhibition around the following disks: ceftazidime, cefepime, amikacin, gentamicin, ceftriaxone, trimethoprim-sulphamethoxazole, ciprofloxacin, ampicillin-sulbactam, imipenem and meropenem. MIC of imipenem and meropenem was >32 µg/ml. MIC of colistin ranged from 0.125 to 2 µg/ml with MIC50 and MIC90 being 0.5 and 2, respectively. Thus, no isolate was found resistant to colistin. MIC of tigecycline also ranged from 0.125 to 2 µg/ml with MIC50 and MIC90 being 0.5 and 2, respectively. Thus, all 30 isolates were XDR.

**Detection of carbapenemase production genes by Multiplex-PCR**

The carbapenem resistant *A. baumannii* (CRAB) isolates examined for the presence of five *bla*OXA genes by PCR showed that *bla*OXA-51 gene was present in all CRAB strains while, 27/30 (90%) isolates were positive for *bla*OXA-23 and 46.6% strains displayed *bla*OXA-24 gene. The *bla*OXA-58 and *bla*OXA-143 genes did not manifest in any of the CRAB strains.

**Quantitative biofilm formation assays**

All *A. baumannii* isolates were able to form varying degrees of biofilm. The mean optical densities for isolates ranged from 0.06 nm to 0.51 nm; however, no significant result was obtained when optical densities were compared with the presence of biofilm genes. Based on the results, biofilm formation capabilities of the isolates were classified weak, moderate, and strong biofilm producer. Of 30 XDR *A. baumannii* isolates, 19/30 (63.3%), 7/30 (23.3%), and 4/30 (13.3%) isolates displayed weak, moderate, and strong adherence activity in the microplate assay, respectively.

**Detection of biofilm, motility, autoinducer synthase and virulence genes by PCR**

Among 30 *A. baumannii* isolates, all were positive for biofilm-associated and autoinducer genes comprising *bfmSR*, *cseE*, *pgaA*, abal and *pda*D. However, detection rates of *bap* and *bla-PER1* were 76.6% (*n*=23) and 83.3% (*n*=25), respectively. However, the mean for biofilm biomass in *bap*, and *blaPER-1* positive isolates were 0.06 nm to 0.37 nm and 0.06 to 0.51 nm respectively. All isolates were also positive for type I *fimbriae*, *PilT* motility related genes, and *ompA* virulence gene.

**Tri-locus sequence typing**

Sequence-based typing revealed all isolates shared the same combination of alleles at the three loci belonged to only one group 1 (European clone II). No isolate belonged to Group 2 or Group 3 or any new variant.

**PCR-based replicon typing (AB-PBRT) method**

After analyzing the presence of *rep* genes, the results showed that *rep6* and *rep2* genes had highest frequency (75% and 65%, respectively), followed by *rep3*, *rep4*, *rep5*, *rep17* with 15%, 37%, 3%, 50% frequency, respectively. No isolate belonged to replicase groups 1, 7-16, 18, and 19. Figs 1 and 2 depicts the genetic characterization of predominant *rep 6* and *rep 2* positive *A. baumannii* isolates. Table 3 depicts the comparative result of Tri-locus sequence typing and PCR-based replicon typing methods utilized for typing the XDR *A. baumannii* clinical isolates.

**DISCUSSION**

Initially, *A. baumannii* emerged as an opportunistic pathogen and the treatment of infection was managed with β-lactams. The persistent presence of *A. baumannii* in the hospital setting allowed it to encounter antibiotics which lead to the emergence of successful clones with particular antibiotic resistance characteristics.43 Currently, *A. baumannii* marks the culmination of organisms facing antibiotic resistance, the so-called paralyzing situation in therapeutics. The organism is notorious for causing serious infections in intensive care units (ICUs).44 In our study, 73.3% of clinical isolates were obtained from ICUs. Unfortunately, all isolates were resistant to antibiotics commonly used by infectious specialist in our hospital setting except colistin and tigecycline. Earlier research studies displays this organism as MDR45 while, later published research studies marked them as XDR46 or PDR.47 More than 90% clinical isolates were resistant to ceftazidime, cefotaxime, cefepime, amikacin, ciprofloxacin, piperacillin-tazobactam, ampicillin-sulbactam and co-tri-
moxazol in an Iranian study performed on clinical isolates obtained from two hospitals.44

Carbapenem was once considered as an effective drug for the treatment of infections caused by A. baumannii, but in recent decades, the rate of carbapenem-resistant A. baumannii strains has increased dramatically and is spreading throughout the world.48-51 Studies from Asian countries including China, Thailand, and Taiwan52-54, show carbapenem resistance to vary from 40 to 60%. In general, carbapenem-hydrolyzing class D β-lactamases (CHDLs) in A. baumannii is mainly mediated by $bla_{OXA-23}$ and $bla_{OXA-51-like}$. $bla_{OXA-23}$ are the most common OXA type of carbapenem resistance in Iran.56 In the present investigation performed on XDR strains, all isolates had $bla_{OXA-51-like}$ gene while the frequency of $bla_{OXA-23}$ and $bla_{OXA-24}$ was 90% and 46.6%, respectively. We did not find presence of $bla_{OXA-58}$ and $bla_{OXA-141}$ genes in any isolate. In most studies performed on CHDLs, $bla_{OXA-58}$ gene has not been found57,58 while, $bla_{OXA-23}$ has been reported as an abundant CHDLs, with a frequency of over 80%.59

A study of samples collected from 1991 to 2011 showed that the content of the $bla_{OXA}$ gene in A. baumannii changed from $bla_{OXA-24/40}$ and $bla_{OXA-58}$ to $bla_{OXA-23}$. Since 2008, the $bla_{OXA-23}$ gene has been the most abundant type of OXA among Acinetobacter baumannii species around the world. The study also showed that with the change in OXA content, the strains' ability to bind and form biofilm has decreased.61 The results of our studies and studies in other parts of the world and in Iran show that this bacterium is resistant to carbapenem. As usage of carbapenem is the last resort treatment strategy, it is of great concern.

According to the results of the present investigation, 13.3% XDR A. baumannii strains had potentiality to form strong biofilm while other strains produced either moderate (23.3%) or weak (63.3%) biofilm. No significant relationship was observed between resistance and biofilm formation among A. baumannii species in our study. Contrary to the phenotypic findings, we could observe presence of biofilm genes in all A. baumannii isolates except bap and blaPER genes, which were not present in all strains. Study conducted in China10 did not indicate a direct relationship between biofilm formation and increased antibiotic resistance, but could show a balance between the two so that weak and strong biofilm strains had the same resistance level. In addition, biofilm production was proposed as a mechanism for survival in strains that have less resistance. Another study also found no difference in biofilm formation in MDR and non-MDR strains.61 By contrast, a strong association between biofilm formation and drug resistance has been reported in an Iranian study.62 An Iranian study indicated high prevalence of ompA, csuE and bfmSR genes in XDR strains.63 Compatible to another studies62,64,65 we found the frequency of ompA and csuE genes to be much greater than bap gene. All A. baumannii strains harboured pgaD and abal genes in contrast to another study conducted in Iran, where quiet low frequency of pgaD and abal genes were reported (45.3% and 14%, respectively). This difference may be due to XDR strains in our study. Studies have shown that the abal gene, an autoinducer synthase, is involved in the late stages of biofilm formation, and mutation of this gene disrupts the biofilm formation process.14 Since in our research study, all A. baumannii strains were positive for at least one of the biofilm-associated genes thus, abal may be positive in all strains. In another Iranian study, the abal gene frequency was 18%. Higher frequency in our study may be due to inclusion of only XDR strains; nevertheless, we did not find any relation between biofilm

Figure 1. Genetic characterization of Group 6 replicon positive XDR A. baumannii strains.

Figure 2. Genetic characterization of Group 2 replicon positive XDR A. baumannii strains.
| A. baumannii (AB) isolates | Tri-locus sequence typing and SGs (sequence groups) | Replicase typing and replicase GR (groups) and genes |
|---------------------------|---------------------------------------------------|---------------------------------------------------|
|                           | SG1 | SG2 | SG3 | GR2 | GR3 | GR4 | GR5 | GR6 | GR17 |
| AB 1                      | +   | -   | -   | -   | +   | -   | -   | +   | -    |
| AB 2                      | +   | -   | -   | +   | -   | -   | -   | -   | -    |
| AB 3                      | +   | -   | -   | -   | +   | -   | -   | -   | +    |
| AB 4                      | +   | -   | -   | -   | +   | -   | -   | +   | -    |
| AB 5                      | +   | -   | -   | -   | -   | -   | -   | -   | +    |
| AB 6                      | +   | -   | -   | -   | -   | -   | -   | -   | +    |
| AB 7                      | +   | -   | -   | +   | -   | -   | +   | -   | -    |
| AB 8                      | +   | -   | -   | +   | -   | -   | -   | -   | +    |
| AB 9                      | +   | -   | -   | +   | -   | -   | -   | -   | -    |
| AB 10                     | +   | -   | -   | -   | -   | +   | -   | +   | +    |
| AB 11                     | +   | -   | -   | +   | -   | -   | -   | -   | +    |
| AB 12                     | +   | -   | -   | +   | -   | -   | -   | -   | +    |
| AB 13                     | +   | -   | -   | -   | -   | +   | -   | -   | +    |
| AB 14                     | +   | -   | -   | +   | +   | +   | -   | +   | -    |
| AB 15                     | +   | -   | -   | -   | -   | +   | +   | -   | -    |
| AB 16                     | +   | -   | -   | -   | +   | -   | -   | -   | -    |
| AB 17                     | +   | -   | -   | +   | +   | +   | -   | -   | -    |
| AB 18                     | +   | -   | -   | -   | -   | +   | -   | -   | +    |
| AB 19                     | +   | -   | -   | +   | -   | -   | -   | -   | +    |
| AB 20                     | +   | -   | -   | -   | -   | +   | -   | -   | +    |
| AB 21                     | +   | -   | -   | -   | +   | +   | -   | -   | -    |
| AB 22                     | +   | -   | -   | +   | +   | +   | -   | -   | -    |
| AB 23                     | +   | -   | -   | -   | -   | -   | +   | -   | +    |
| AB 24                     | +   | -   | -   | -   | -   | -   | +   | -   | +    |
| AB 25                     | +   | -   | -   | +   | +   | +   | -   | -   | -    |
| AB 26                     | +   | -   | -   | -   | -   | -   | -   | +   | +    |
| AB 27                     | +   | -   | -   | -   | -   | -   | -   | -   | +    |
| AB 28                     | +   | -   | -   | +   | -   | -   | +   | -   | -    |
| AB 29                     | +   | -   | -   | -   | +   | -   | +   | -   | -    |
| AB 30                     | +   | -   | -   | -   | +   | -   | -   | -   | -    |

Table 3. Comparative result of Tri-locus sequence typing and replicase typing methods for XDR A. baumannii clinical isolates

production and abaI gene. In the present investigation, all A. baumannii isolates were XDR and biofilm producers, which is a clinical apprehension. The frequency of abaI gene was 59.8% in a Chinese study but the isolates were not specifically XDR. In a study performed in Iraq, 66% non-XDR isolates revealed presence of abaI gene and all these isolates were biofilm producers. Based on the results of this study and other investigations, we can conclude that though abaI gene is related to autoinducer synthesis, it plays an important role in biofilm production and is associated with antibiotic resistance. Despite progress in the antibiotic resistance mechanisms in A. baumannii, still knowledge is scarce in understanding the genetic factors that have driven the recent evolution of A. baumannii toward multidrug resistance. A. baumannii may develop resistance to carbapenems through plasmid-mediated acquisition of carbapenem-hydrolyzing class D β-lactamases (CHDLs). In particular the blaOXA-58 and blaOXA-23 genes, encoding the OXA-58 and OXA-23 CHDLs respectively, have been reported from A. baumannii isolates collected from distant parts of the world in association with plasmids. In the present study blaOXA-51 was present in all A. baumannii CHDLs producing strains and blaOXA-23 was observed in 90% strains while, the blaOXA-58 was not detected in any isolate. In the present study, XDR A. baumannii were investig-
ed by plasmid replicon typing and more than 60% A. baumannii strains belonged to rep6 and rep2 types. This suggests diffusion of the carbapenem-hydrolyzing oxacillinase genes bla\textsubscript{OXA-23} and bla\textsubscript{OXA-58}. Bap, known to be the sources of resistance to carbapenems in A. baumannii strains, are related to two multiple plasmid types.

Compatible results were obtained on 3LST multiplex PCR typing, where all A. baumannii strains belonged to sequence group SG1, corresponding to international clone II. Studies reported from Spain and Greece shows 100% and 99.4% A. baumannii isolates belonged to international clone II, respectively.\textsuperscript{30,70} The results of our research also indicate that SG1 is more common among other groups. Recent study have shown that plasmids carrying these genes may be due to the high prevalence of carbapenem resistance through CHDLs.\textsuperscript{27} Prior to our study, Acinetobacter baumannii typing had not been performed using replicase typing in Iran. In a study conducted in Italy, A. baumannii strains belonged to groups 6 and 2, with a frequency of 96.8% and 70%, respectively. Also, the results of these studies have shown that rep 6 may play a major role in the horizontal transmission of resistance among members of this species.\textsuperscript{71} Because we did not perform transferability experiment, the genetic dissemination cannot be proved. However, our results showed predominance of rep 6 and rep 2 genes in XDR A. baumannii strains.

**CONCLUSIONS**

The results of our study shows that we are facing a jeopardous situation. Presence of biofilm associated genes except bla\textsubscript{PER-1} and Bap and other virulence genes in all clinical isolates is a feature which confirms the endemcity of A. baumannii and appraises the nosocomial nature of the bacteria. Our investigation showed that all A. baumannii strains belonged to SG1 group and two major replicase groups, which further highlights an emergence of one type of clone and its dissemination. It is thus critical to work on their transfer ability and prevention of resistant bacterial dissemination in order to avoid further restrictions on therapeutic options. In order to evaluate new specific interventions, it is essential to gather specific data on the antibiotic resistance in A. baumannii. As antibiotics are gradually losing their effectiveness today, other ways such as prevention of biofilm formation or using quorum-sensing quenchers should be exploited.

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**Author Disclosure Statement**

No competing financial interests exist.

**REFERENCES**

1. Towner K. Acinetobacter: an old friend, but a new enemy. J Hosp Infect 2009; 73(4):355–63.
2. Morris FC, Dexter C, Kostoulas X, et al. The mechanisms of disease caused by Acinetobacter baumannii. Front Microbiol 2019; 10:1601.
3. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 2007; 5(12):939.
4. Ramirez MS, Bonomo RA, Tolmasky ME. Carbapenemases: transforming Acinetobacter baumannii into a yet more dangerous menace. Biomolecules 2020; 10(5):720.
5. World Health Organization (WHO). Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and Pseudomonas aeruginosa in health care facilities. Available from: https://www.who.int/infection-prevention/publications/guidelines-cre/en/ Genova: World Health Organization; 2017
6. PelegAY, Seifert H, Paterson DL. Acinetobacter baumannii emergence of a successful pathogen. Clin Microbiol Rev 2008; 21(3):538–82.
7. Fu Y, Jiang J, Zhou H, et al. Characterization of a novel plasmid type and various genetic contexts of blaOXA-58 in Acinetobacter spp. from multiple cities in China. PLoS One 2014; 9(1):e84680.
8. Saijriya K, Swathi C, Ratnakar K, et al. Quorum-sensing system in Acinetobacter baumannii: a potential target for new drug development. J Appl Microbiol 2020; 128(1):15–27.
9. Castillo-Juarez I, Lopez-Jacome LE, Soberón-Chávez G, et al. Exploiting quorum sensing inhibition for the control of Pseudomonas aeruginosa and Acinetobacter baumannii biofilms. Curr Top Med Chem 2017; 17(17):1915–27.
10. Qi L, Li H, Zhang C, et al. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in Acinetobacter baumannii. Front Microbiol 2016; 7:483.
11. Azizi O, Shakhbaze MR, Modarresi F, et al. Molecular detection of class-D OXA carbapenemase genes in biofilm and non-biofilm forming clinical isolates of Acinetobacter baumannii. Jundishapur J Microbiol 2015; 8(1):e21042.
12. Farshadzadeh Z, Hashemi FB, Rahimi S, et al. Wide distribution of carbapenem resistant Acinetobacter baumannii in burns patients in Iran. Front Microbiol 2015; 6:1146.
13. Gurung J, Khuriem BA, Banik A, et al. Association of biofilm production with multidrug resistance among clinical isolates of Acinetobacter baumannii. Front Microbiol 2017; 8:2394.
14. Farshadzadeh Z, Hashemi FB, Rahimi S, et al. Wide distribution of carbapenem resistant Acinetobacter baumannii in burns patients in Iran. Front Microbiol 2015; 6:1146.
Genetic Analysis of XDR A. Baumannii

SD, Weisfeld AS, editors. 13th ed. St. Louis, Missouri: Elsevier; 2014:254–359.

Turton JF; Woodford N, Glover J, et al. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 2006; 44(8):2974–6.

La Scola B, Gundi VA, Khannis A, et al. Sequencing of the rpoB gene and flanking spacers for molecular identification of Acinetobacter species. J Clin Microbiol 2006; 44(3):827–32.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 27th edition [M100-S27]: CLSI, Wayne, PA; 2017 |

Gomaa F, Helal Z, Khan M. High prevalence of blaNDM-1, blaVIM, qacE, and qacEΔ1 genes and their association with decreased susceptibility to antibiotics and common hospital bicldes in clinical isolates of Acinetobacter baumannii. Microorganisms 2017; 5(2):18.

Karaag upward poulous DE, Kelesidis T, Kelesidis I, et al. Tigecycline for the treatment of multidrug-resistant (including carbapenem-resistanct) Acinetobacter infections: a review of the scientific evidence. J Antimicrob Chemother 2008; 62(1):45–55.

Curdio D, Fernández F. Tigecycline disk diffusion breakpoints of Acinetobacter spp.: a clinical point of view. J Clin Microbiol 2007; 45(6):2095–6.

Woodford N, Ellington MJ, Coelho JM, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006; 27(4):351–3.

Badave GK, Kulkarni D. Biofilm producing multidrug resistant Acinetobacter baumannii: an emerging challenge. J Clin Diagn Res 2015; 9(1):DC08.

Farshadzadeh Z, Taheri B, Rahimii S, et al. Growth rate and biofilm formation ability of clinical and laboratory-evolved colistin-resistant strains of Acinetobacter baumannii. Front Microbiol 2018; 9:153.

Hatami R. The frequency of multidrug-resistance and extensively drug-resistant Acinetobacter baumannii in west of Iran. J Clin Microbiol Infect Dis 2018; 1(1):4–8.

Gonzalez-Villoria AM, Valverde-Garduno V. Antibiotic-resistant Acinetobacter baumannii increasing success remains a challenge as a nosocomial pathogen. J Pathog 2016; 2016:7318075.

Fallah F, Noori M, Hashemi A, et al. Prevalence of blaNDM, blaPER, blaVEB, blaIMP, and blaVIM genes among Acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Scientifica 2014; 2014:245162.

Abbo A, Navon-Venezia S, Hammer-Muntz O, et al. Multidrug-resistant Acinetobacter baumannii. Emerging Infect Dis 2005; 11(1):22.

HST H. Emergence of an extreme-drug-resistant (XDR) Acinetobacter baumannii carrying blaOXA-23 in a patient with acute necrotising haemorrhagic pancreatitis. J Hosp Infect 2010; 30:1–2.

Sobouti B, Mirshekar M, Fallah S, et al. Pan drug-resistant Acinetobacter baumannii causing nosocomial infections among burnt children. Med J Islam Repub Iran 2020; 34(1):164–7.

Nowak P, Paluchowska P. Acinetobacter baumannii: biology and drug resistance - role of carbapenemases. Folia Histochem Cytobiol 2016; 54(2):61–74.

Mohgadasi M, Kalantar-Neyestani D, Karam-Zarandi M, et al. Investigation of antimicrobial susceptibility patterns and frequency of bla OXA genes in carbapenem resistant Acinetobacter baumannii strains. Sci J Kurdistan Univ Medical Sci 2018; 23(5):108–19.

Leung EC-m, Leung PH-m, Lai RW-m. Emergence of carbapenem-resistant Acinetobacter baumannii ST195 harboring bla OXA-23
isolated from bacteremia in Hong Kong. Microb Drug Resist 2019; 25(8):1199–203.
51. Watkins RR. A formidable foe: carbapenem-resistant Acinetobacter baumannii and emerging nonantibiotic therapies. Expert Rev Anti Infect Ther 2018; 16(8):591–3.
52. Azimi L, Talebi M, Poursafie M-R, et al. Characterization of carbapenemases in extensively drug resistance Acinetobacter baumannii in a burn care center in Iran. Int J Mol Cell Med 2015; 4(1):46.
53. Lee Y-L, Lu M-C, Shao P-L, et al. Nationwide surveillance of antimicrobial resistance among clinically important Gram-negative bacteria, with an emphasis on carbapenems and colistin: Results from the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) in 2018. Int J Antimicrob Agents 2019; 54(3):318–28.
54. Thapa B, Tribuddharat C, Srifuengfung S, et al. High prevalence of BLA^ sub OXA-23^ in oligoclonal carbapenem-resistant Acinetobacter baumannii from Siriraj hospital, Mahidol University, Bangkok, Thailand. Southeast Asian J Trop Med Public Health 2010; 41(3):625.
55. Royer S, de Campos PA, Araújo BF, et al. Molecular characterization and clonal dynamics of nosocomial blaOXA-23 producing XDR Acinetobacter baumannii. PloS One 2018; 13(6):e0198643.
56. Hsu L-Y, Apsisarthanarak A, Khan E, et al. Carbapenem-resistant Acinetobacter baumannii and Enterobacteriaceae in south and southeast Asia. Clin Microbiol Rev 2017; 30(1):1–22.
57. Zafari M, Feizabadi MM, Jafari S, et al. High prevalence of oxacillinase genes in drug resistant clinical isolates of Acinetobacter baumannii strains in a teaching hospital of Tehran. Acta Microbiol Immunol Hung 2017; 64(4):385–94.
58. Chmielarczyk A, Pilarczyk-Żurek M, Kamińska W, et al. Molecular epidemiology and drug resistance of carbapenem-resistant Acinetobacter baumannii isolated from hospitals in southern Poland: ICU as a risk factor for XDR strains. Microb Drug Resist 2016; 22(4):328–35.
59. Bardbari AM, Arabestani MR, Karami M, et al. Correlation between ability of biofilm formation with their responsible genes and MDR patterns in clinical and environmental Acinetobacter baumannii isolates. Microb Pathog 2017; 108:122–8.
60. Romaini P, Palermo RL, Cavallini JF, et al. Multidrug- and extensively drug-resistant Acinetobacter baumannii in a Tertiary hospital from Brazil: the importance of carbapenemase encoding genes and epidemic clonal complexes in a 10-Year study. Microb Drug Resist 2019; 25(9):1365–73.
61. Thummeepak R, Kongthai P, Leungtongkam U, et al. Distribution of virulence genes involved in biofilm formation in multi-drug resistant Acinetobacter baumannii clinical isolates. Int Microbiol 2016; 19(2):121–9.
62. Fallah A, Rezaee MA, Hasani A, et al. Frequency of bap and cpaA virulence genes in drug resistant clinical isolates of Acinetobacter baumannii and their role in biofilm formation. Iran J Basic Med Sci 2017; 20(8):849.
63. Zeighami H, Valadkhani F, Shapouri R, et al. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumanii isolated from intensive care unit patients. BMC Infect Dis 2019; 19(1):629.
64. Azizi O, Shahcheraghi F, Salimizand H, et al. Molecular analysis and expression of bap gene in biofilm-forming multi-drug-resistant Acinetobacter baumannii. Rep Biochem Mol Biol 2016; 5(1):62.
65. Badmasti F, Siadat SD, Bouzari S, et al. Molecular detection of genes related to biofilm formation in multidrug-resistant Acinetobacter baumannii isolated from clinical settings. J Med Microbiol 2015; 64(5):559–64.
66. Liu H, Wu Y-Q, Chen L-P, et al. Biofilm-related genes: analyses in multi-antibiotic resistant Acinetobacter baumannii isolates from Mainland China. Med Sci Monit 2016; 22:1810.
67. Al-Kadmy I, Ali A, Salman I, et al. Molecular characterization of Acinetobacter baumannii isolated from Iraqi hospital environment. New Microbes New Infect 2018; 21:51–7.
68. Zarrilli R, Vitale D, Di Polpo A, et al. A plasmid-borne blaOXA-58 gene confers imipenem resistance to Acinetobacter baumannii isolates from a Lebanese hospital. Antimicrob Agents Chemother 2008; 52(11):4115–20.
69. Corvec S, Poirel L, Naas T, et al. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in Acinetobacter baumannii. Antimicrob Agents Chemother 2007; 51(4):1350–3.
70. Nafplioti K, Galani I, Angelidis E, et al. Dissemination of international clone II Acinetobacter baumannii strains coproducing OXA-23 carbapenemase and 16S rRNA methylase ArmA in Athens, Greece. Microb Drug Resist 2020; 26(1):9–13.
71. Towner KJ, Evans B, Villa L, et al. Distribution of intrinsic plasmid replicase genes and their association with carbapenem-hydrolyzing class D β-lactamases in European clinical isolates of Acinetobacter baumannii. Antimicrob Agents Chemother 2011; 55(5):2154–9.
72. Gong Y, Shen X, Huang G, et al. Epidemiology and resistance features of Acinetobacter baumannii isolates from the ward environment and patients in the burn ICU of a Chinese hospital. J Microbiol 2016; 54(8):551–8.
Генетическая характеристика Acinetobacter baumannii с широкой лекарственной устойчивостью: невообразимое препятствие

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Резюме

Введение: Инфекции, вызванные Acinetobacter baumannii, вызывают всё большую озабоченность в области общественного здравоохранения. Способность бактерий приобретать устойчивость к ряду широко используемых антибиотиков делает их серьёзным патогеном.

Цель: Молекулярная характеристика клинических изолятов A. baumannii типа экстенсивной лекарственной устойчивости (ЭЛУ) с помощью полимеразной цепной реакции.

Материалы и методы: Тридцать ЭЛУ A. baumannii были протестированы на наличие генов, кодирующих устойчивость к карбапенемазе, объём биоплёнки, аутоиндуктивную синтазу, вирулентность и подвижность поверхности с помощью полимеразной цепной реакции (ПЦР). Затем тип изолятов определяли с помощью репликонов на основе плазмид (Rep) (PBRT) и типирования трилокусной последовательности.

Результаты: Во всех 30 штаммах XDR A. baumannii были идентифицированы гены, связанные с поверхностной подвижностью, аутоиндуктивной синтазой, детерминантой вирулентности, гены, связанные с биоплёнкой, за исключением PER и bap, с частотами 83.3% и 76.6% соответственно. Анализ генов rep показал наибольшую частоту генов rep6 и rep2 с частотой 75% и 65% соответственно. Все штаммы ЭЛУ A. baumannii относятся к группе SG I (European clone II).

Заключение: Наши результаты выявили необычную пластичность A. baumannii ЭЛУ и утверждают, что эти штаммы стали эндемичными в нашей больнице, что может вызвать опасения в ближайшем будущем.

Ключевые слова

Acinetobacter baumannii, аутоиндуктор-синтаза, биоплёнка, гидролизующие карбапенем β-лактамазы класса D, обширная лекарственная устойчивость, типирование репликаны, типирование трилокозной последовательности, вирулентность