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Antibiotic Resistance Pattern and Prevalence of *bla*$_{OXA-51}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{CTX}$, *tetA* and *tetB* Genes in *Acinetobacter baumannii* Isolated from Clinical Specimens of Hospitals in Tabriz city, Iran

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Abstract

Background: Nowadays, the resistance of pathogenic bacteria to antibiotics has become a global problem. *Acinetobacter baumannii* is an important opportunistic nosocomial pathogen. *Acinetobacter baumannii* plays a significant role in antibiotic resistance.
Objectives: The purpose of this study was to investigate the prevalence of the blaOXA-51, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{CTX-M}$, *tetA* and *tetB* genes and antibiotic resistance pattern of *A. baumannii* isolated from hospitals in Tabriz city, Iran.
Methods: This study was descriptive cross-sectional research, performed on 129 isolates of Acinetobacter from different clinical specimens. The Isolates were identified using standard laboratory methods and culture in selective mediums. The antibiotic resistance pattern of isolates was also determined by the Kirby-Bauer disk diffusion susceptibility test. Phenotypic and genotypic detection of blaOXA-51, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{CTX-M}$, *tetA* and *tetB* genes in the isolates was carried out by a combined disk test (CDT) and polymerase chain reaction (PCR), respectively.
Results: The highest resistance of isolates was determined to cefotaxime (100%) and ceftazidime (100%). The results of CDT showed that 14 (12.96%) isolates could produce extended-spectrum Beta-lactamases (ESBLs). However, the PCR results *bla*$_{OXA-51}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{CTX-M}$, *tetA* and *tetB* genes showed that these genes were in 100%, 18.51%, 16.66%, 32.40%, 16.66%, 31.48%, 32.40% and 21.29% of isolates, respectively.
Conclusions: Due to the high prevalence of antimicrobial resistance in strains, rapid and timely detection of antibiotic-resistant *A. baumannii* strains is necessary for the selection of an appropriate therapeutic approach and prevention of their prevalence.

Keywords: *Acinetobacter baumannii*, Beta-lactamase Gene, Antibiotic Resistance, PCR

1. Background

Acinetobacter spp. are obligate aerobic, oxidase-negative, non-motile, Gram-negative coccobacilli and opportunistic pathogens that can be easily isolated from soil and water, and sometimes from the hospital environments (1, 2). They don't require a specific nutrient medium to survive. *Acinetobacter* spp. grow easily in conventional laboratory mediums without any pigment (3, 4). Due to its low nutritional requirements for growth, this bacterium can survive for long periods in adverse conditions, dry surfaces as well as in aquatic environments (5). *Acinetobacter baumannii* is one of the most common pathogenic bacteria causing nosocomial infections in patients hospitalized in intensive care units. People with neutropenia, cystic fibrosis, and immune deficiency are exposed to the risk of infection with *A. baumannii* (6). The catheter and other medical equipment may lead to the outbreak of this bacterium in the hospital (7). Owing to the high levels of antibiotic resistance in comparison with other nosocomial isolates and its high prevalence in hospital environments, *A. baumannii* is known as an important cause of disease, mortality and economic loss in different countries (8, 9). Besides respiratory tract infections, *A. baumannii* may also responsible for urinary tract and wound infections in hospitals (10). The resistance of *A. baumannii* strains to antibiotics can be intrinsic or through the obtaining of genetic factors. Most of them are resistant to ampicillin, amoxicillin/clavulanic acid, antistaphylococcal penicillin, extended-spectrum cephalosporins (except ceftazidime and cefepime), tetracycline, macrolides,

rifampicin and chloramphenicol (11, 12). As reported in most hospitals worldwide, multidrug-resistant (MDR) *A. baumannii* has recently become a major concern in hospitals (13). The prevalence of MDR *A. baumannii* to other sites indicates the role of this organism in the rapid spread of resistance genes (14-16). Beta-lactamases are inactivating enzymes for the beta-lactam antibiotics. The first identified beta-lactamase is penicillinase (17-21). *CTX-M* type beta-lactamases, firstly identified in Germany in 1989, consist of a group of Extended-spectrum β-lactamases (ESBLs) encoded by the plasmid (22). Based on the amino acid sequences, *CTX-M* beta-lactamases are classified into five major groups: *CTX-M1*, *CTX-M2*, *CTX-M8*, *CTX-M9*, and *CTX-M25* (23). The *CTX-M* enzyme mainly hydrolyzes cefotaxime and often has poor activity against ceftazidime. However, *CTX-M15* has a strong activity against ceftazidime (24). The prevalence of ESBLs, especially *CTX-M*, has increased in recent years (25). These enzymes result in the resistance of bacteria to penicillin and a wide range of third-generation cephalosporins. However, ESBLs are sensitive to several antibiotics such as cephamycin and carbapenem. Also, some of the antibiotics such as clavulanic acid, tazobactam and sulbactam completely inhibit these enzymes (26). *Acinetobacter baumannii* is inherently capable of producing class D oxacillins (belonging to the OXA-51 group of enzymes) and non-induced AmpC cephaloporinases (27). The bla_{PER-1} gene was first found in *Pseudomonas aeruginosa*. It has since been widely observed in Acinetobacter (28). The bla_{VEB-1} gene was first observed during the outbreak of clonal strains of *A. baumannii* in the ICU of a French hospital (29). Pumping of the drug out of the bacteria by efflux mechanisms is also related to the specificity of MDR. The *tetA* is involved in resistance to tetracycline and *tetB* is involved in the minocycline pump in addition to tetracycline (27, 30). VIM-type beta-lactamase in Acinetobacter species was first observed in Europe and then reported worldwide (31, 32). NDM beta-lactamase is a transmissible class B molecular β-lactamase recently identified in New Delhi, India (33). Beta-lactam resistance genes in *A. baumannii* are usually located on mobile genetic elements and therefore can easily transmit between different strains. Thus, identification of the ESBLs-producing strains can be an essential step in the treatment of their infections.

2. Objectives

Due to the high prevalence of antibiotic resistance genes and nosocomial infections, the aim of the present study was to isolate *A. baumannii* from clinical specimens and determine the prevalence rate of bla_{OXA-51}, bla_{NDM}, bla_{VIM}, bla_{PER}, bla_{VEB}, bla_{CTX-M}, *tetA* and *tetB* genes in the isolates.

3. Methods

This study was descriptive cross-sectional research performed on 129 clinical isolates of Acinetobacter isolated from blood, urine, wound exudates and respiratory secretions in the hospitals in Tabriz city from March to December 2019. All of the isolates were detected using standard laboratory tests, microbiological experiments and differential biochemical tests including oxidase, catalase, urease, oxidation-fermentation (OF), Triple Sugar Iron (TSI) tests, culture in Simmons citrate agar, sulfur indole motility media (SIM), Methyl red-Voges-Proskauer (MR-VP) broth as well as, the growth in 37°C and 42°C. Antibiotic resistance patterns of *A. baumannii* isolates were determined by agar disc diffusion method based on the guidelines of the clinical & laboratory standards institute (CLSI) (34). The used antibiotic disks are included: ceftazidime (30 μg), ciprofloxacin (5 μg), levofloxacin (10 μg), cefotaxime (30 μg), Aztreonam (30 μg), cefepime (30 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), meropenem (10 μg), piperacillin/tazobactam (10/100 μg), tetracycline (30 μg)) and polymyxin B (300 μg) (Mast Diagnostics Mast group Ltd., Merseyside, UK). The standard strain of *A. baumannii* ATCC 19606 and *Escherichia coli* ATCC 25922 was used as positive and negative controls, respectively. The ESBL-producing isolates were identified by combined disk test using ceftazidime (30 μg), cefotaxime (30 μg), ceftazidime/clavulanic acid (30 μg/10 μg) and cefotaxime/clavulanic acid (30μg/10μg). After incubation at 37°C for 24 hours, ESBL-producing isolates had a zone of inhibition with a diameter $\geq$ of 5 mm around ceftazidime/clavulanic acid discs in comparison with ceftazidime or cefotaxime discs (35). The DNA samples were extracted from the isolates using a commercial kit (Invitek, STRATEC Molecular-Germany). To identify the bla_{OXA-51}, bla_{NDM}, bla_{VIM}, bla_{PER}, bla_{VEB}, bla_{CTX-M}, *tetA* and *tetB* genes in the isolates, the polymerase chain reaction (PCR) technique was performed using the specific primers (Table 1). The reaction was performed in a total volume of 20 μL, including 10 μL of Master Mix (Amplicon Denmark), 2 μL of template DNA, 10 pM of primer and distilled water. The heating program in thermocycler was as follows: one cycle of initial denaturation at 94°C for 3 min, 35 cycles of denaturation in 35°C for 30 sec, annealing at 45°C for 1 minute, and extension at 72°C for 1 min. The final extension was carried out at 72°C for 4 minutes. The PCR products were run on 1% agarose gel in TBE buffer for 60 min at 100°C. The gel was then placed in a tank containing ethidium bromide for 15 minutes. The results were visualized using a gel documentation system under the UV light. Standard strains of *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as quality control according to

the CLSI.

4. Results

Out of 129 isolates, 108 (83.72%) isolates were identified as *A. baumannii*. The mean age of patients with *A. baumannii* infections was 52 $\pm$ 24.4 years. The organism was isolated from 58 (53.7%) of urine cultures, 10 (9.26%) of wound discharges, 34 (31.48%) of blood cultures and 6 (5.56%) of respiratory tract secretions. The isolates showed the least resistance to polymyxin B (47.22%). The highest resistance of them was detected to cefotaxime (100%) and ceftazidime (100%) (Figure 1). Out of 108 isolates, 103 isolates (95.37%) were identified as MDR (resistant to more than three classes of antibiotics). The results of the combined disk test showed that 14 (12.96%) isolates were ESBL-positive. PCR results for target genes showed that 43 (39.81%) isolates contained *tetA* gene, 35 (32.40%) isolates contained bla_{PER-1} gene, 34 (31.48%) isolates contained bla_{CTX-M} gene, 23 (21.29%) isolates contained *tetB* gene, 20 (18.51%) isolates contained *blaNDM* gene,18 (16.66%) isolates contained bla_{VEB-1} gene and 18 (16.66%) isolates contained bla_{VIM} gene. The *blaOXA-51* gene was positive as a genetic marker for the diagnosis of *A. baumannii* in all isolates. The ESBL-producing isolates showed the highest resistance to the used antibiotic discs among others.

5. Discussion

Production of beta-lactamases by gram-negative bacteria is one of the main mechanisms responsible for their resistance to beta-lactam antibiotics. Since the beta-lactams have wide clinical applications, beta-lactamases have evolved concurrently and played a major role in the failure of antibiotic therapy (43). In the last fifteen years, epidemics of infection caused by beta-lactamase-producing organisms have occurred around the world. So, these enzymes are known as a major threat to the use of cephalosporins. It has also been well established that the treatment of such cephalosporin-resistant infections will not be satisfactory, and the mortality caused by ESBL-producing bacteria is significantly high (44). The emergence and prevalence of ESBL-producing bacteria appear to be due to the widespread use of extended-spectrum beta-lactams. The prevalence of these bacteria in different parts of the hospital has been increased in recent years. In the present study, out of the 129 Acinetobacter isolates, 108 (83.72%) were identified as *A. baumannii*. This result is almost similar to the findings of Nazari Monazam et al. (45) (76.9%), Constantiniu et al. (46) (71%) and Rit et al. (47) (74.02%). However, Ahmadikia et al. (48) reported higher rates (93.1%) than of the present study. In this study, the antimicrobial resistance analysis indicated that all isolates were resistant to ceftazidime and cefotaxime. Ayan et al. (49) found that all 52 isolates were resistant to piperacillin/tazobactam, cefepime, cefotaxime, ceftazidime, gentamicin, and aztreonam. The resistance to aminofloxacin and tetracycline were reported in 8% and 74% of strains, respectively. Biendo et al., in a study about the antibiotyping of *A. baumannii* isolates, found that 15 of 18 isolates were resistant to ticarcillin, ticarasilamine/clavulanic acid, piperacillin/tazobactam, ceftazidime and aztreonam (50). Their findings are quite consistent with the results of the present study. In the study of Smolyakov et al., (51) and Wang et al., (52), all strains were resistant to imipenem. Also, Saadatian (2005) reported that 95.5% of *A. baumannii* isolates were resistant to amikacin. These results were is consistent with the findings of the present study (53). In the present study, the resistance level of *A. baumannii* isolates to polymyxin B was high, so that 47.22% of isolates were resistant to this antibiotic. Polymyxins are the last-line treatment for MDR isolates of *A. baumannii*. Therefore, treatment of *A. baumannii* infections, which is resistant to these antibiotics, is very difficult (54). In the present study, 103 isolates (95.37%) had MDR that was higher than the findings of Joshi (55) (75%) and Bahador (56) (45%) and less than Ahmadikiya (98.9%) (48). In this study, the prevalence of ESBL-producing *A. baumannii* in clinical specimens was 12.96%, which is higher than the results of Ahmadikiya (48) and lower than the findings of Sinha (57) in India and Maleki (58) in Shiraz. Ranjbar and Farahani in their study of 163 strains of *A. baumannii* showed that 52.2% of the samples were ESBL positive. Which is more than the findings of the present study (59). In the study of Ahmadikiya et al. (48), 31.6% of isolates were positive for the bla_{CTX-M} gene, which is similar to the findings of present study. Also, in the study of Shahcheraghi et al. (60) and Celenza et al. (61), these rates were 1.2% and 30.4%, respectively, which are lower than the findings of the present study. In the present study, the presence of the bla_{OXA-51}, bla_{NDM}, bla_{VIM}, bla_{PER}, bla_{VEB}, bla_{CTX-M}, *tetA* and *tetB* genes are 100%, 18.51%, 16.66%, 32.40%, 16.66%, 31.48%, 32.40% and 21.29%, respectively. Safari et al reported that 58% and 20% of ESBL-positive A. baumannii isolates contained SHV and CTX-M genes, respectively (62). Goudarzi et al. In 2016, the resistance of isolated strains to tested antibiotics was 95.4% to ceftazidime, 100% to cefotaxime, 95.7% to cefepime, 91.7%to imipenem, 91.7% to meropenem, 80.6% to amikacin, 97.2%to piperacillin, 92.6%to ciprofloxacin, 95.4% to piperacillin/tazobactam, 40.7% to gentamicin, 98.1% to ampicillin/sulbactam and 98.1% to co-trimoxazole respectively. PCR results showed that 44.17% of the isolates had bla_{VIM} gene and bla_{NDM} gene was not seen in the strains

Table 1. Sequence of Primers Used

Genes	Sequences	Amplicon Size	References
bla_{OXA-51}	5′-TAA TGC TTT GAT CGG CCT TG-3′	353bp	(36)
	5′-TGG ATT GCA CTT CAT CTT GG-3′		
bla_{NDM}	5'-GGTTTGGCGATCTGGTTTTC-3'	621bp	(37)
	5'-CGGAATGGCTCATCACGATC-3'		
bla_{VIM}	5'-ATTGGTCTATTTGACCGCGTC-3'	514bp	(38)
	5'-AATGCGCAGCACCAGGATAG-3'		
bla_{PER}	5'-GTTAATTTGGGCTTAGGGCAG-3'	855bp	(39)
	5'-CAGCGCAATCCCCACTGT-3'		
bla_{VEB}	5'-CGACTTCCATTTCCCGATGC-3'	643bp	(40)
	5'-GGACTCTGCAACAAATACGC-3'		
bla_{CTX-M}	5'-CGCTTTGCGATGTGCAG-3'	550bp	(41)
	5'-ACCGCGATATCGTTGGT-3'		
tetA	5'-GCGCGATCTGGTTCACTCG-3'	164 bp	(42)
	5'-AGTCGACAGYRGCGCCGGC-3'		
tetB	5'-TACGTGAATTTATTGCTTCGG-3'	206 bp	(42)
	5'-ATACAGCATCCAAAGCGCAC-3'		

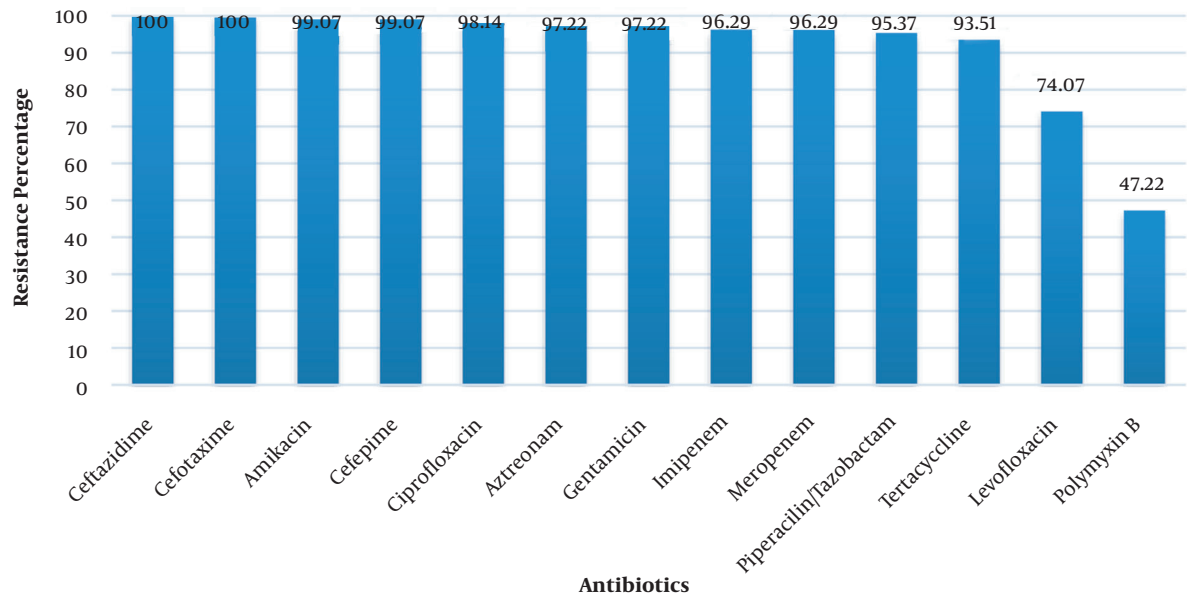

Figure 1. Resistance percentage of *Acinetobacter baumannii* isolates to different antibiotics

(63). Mohammadi et al. Showed in a study that antibiotic resistance in 100 isolates of *A. baumannii* was related to antibiotics: Cefimoimide (97%), Ceftriaxone (95%), Amikacin (95%), Imipenem (76%), Piperacillin-tazobactam (70%), Meropenem (69%), Gentamicin (63%), Tobramycin (56%), Tetracycline (51%), and Ampicillin-Sulbactam (49%) and lowest resistance was related to polymyxin B. PCR results showed that 17% and 20% of the strains carried bla_{VIM} and bla_{NDM} genes, respectively (64). Azizi and Shahcheraghi during a study in 2017 in Tehran hospi-

tals, showed that all samples were resistant to gentamicin, ciprofloxacin, piperacillin, cefotaxime, ceftazidime and tetracycline. Also, all isolates were identified as resistant to several antibiotics. The *tetA*, *tetB*, bla_{VEB}, bla_{CTX-M} and bla_{PER} genes were identified as 75.3%, 43%, 35.3%, 76.9% and 61.5% of the isolates, respectively (65). In a 2012 study by Asadollahi et al., The prevalence of *tetA* and *tetB* genes was reported to be 95.5% and 65%, respectively (66). The bla_{OXA-51} gene is located on the chromosome in *A. baumannii*. The enzyme *OXA-51* has poor carbapenemase activity, but the addition of the ISAba 1 complement sequence at the 5 'end of the bla_{OXA-51} gene leads to its high expression, and this increase in expression causes resistance to carbapenem (67). In 2015, Badmasti et al reported a 44% presence of the bla_{PER} gene in *A. baumannii* isolates. Which is more than the findings of the present study (68). Ranjbar and Farahani during a study in 2019, the prevalence of bla_{OXA-23}, bla_{VIM}, bla_{PER-1} and *tetB* genes was reported to be 85.1%, 60.5%, 42.3% and 67.8%, respectively (59). The differences between the findings of the present study and other researchers may be due to differences in the place of sample collection, the number of samples studied and even the decrease or increase in antibiotic use in the patients studied

5.1. Conclusions

According to the results of this study, the resistance of *A. baumannii* isolates to various antibiotics especially betalactams is an important therapeutic problem. Also, the production of ESBLs is a major threat for use of extended-spectrum cephalosporins. Therefore, in order to treat infections that are suspected for ESBL production, the appropriate antibiotic should be selected based on the results of the antibiogram test. The findings of the present study indicate the need for making the right decision about the reasonable administration of drugs as well as using novel diagnostic methods in microbiology laboratories.

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Footnotes

Authors' Contribution: AJS and SN conceived the project and designed the experiments. AJS and SN collected the samples. AJS and SN performed laboratory experiments. AJS, HBB and MS analyzed the data. AJS and MS wrote the draft. All authors reviewed and approved the final manuscript.

Conflict of Interests: The authors declare that they have no competing interests.

Data Reproducibility: The data presented in this study are openly available in one of the repositories or will be available on request from the corresponding author by this journal representative at any time during submission or after publication. Otherwise, all consequences of possible withdrawal or future retraction will be with the corresponding author.

Ethical Approval: This study was approved by the Ahar Branch, Islamic Azad University, Ahar, Iran (no registered code). All procedures performed in studies including human participants were according to the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Informed Consent: All patients were informed that their test results may be used anonymously for research purposes and the written informed consent was taken from all of them. For children under the legal age, consent was obtained from their parents or legal protector.

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