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Original Article

Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among *Acinetobacter baumannii* strains isolated from patients admitted to al-Yarmouk hospital

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ABSTRACT

Emerging antibiotic resistance in microorganisms particularly multidrug-resistant bacterial strains are increasing because of misusing antibiotics as well as the evolution of antibiotic resistance mechanisms in new strains. In this regard, *Acinetobacter baumannii* is one of the six most common multidrug-resistant microorganisms related to nosocomial diseases. Recently, carbapenems, as common antibiotics to treat infections caused by *Acinetobacter* have not shown an acceptable efficiency because of the resistance emergence to carbapenems in many strains of this bacterium. In this study, resistant strains of *A. baumannii* were isolated and identified as an appropriate preventive strategy to reduce bacterial infections in al-yarmouk hospital of Iraq. Disc diffusion test and PCR method were used to isolate of resistant strains and identify beta-lactamase genes of blaAmpC, blaTEM, blaVIM, and blaSHV. This study showed that these genes were contributed to the antibiotic resistance with about 18.4% and ≥53.5% strains expressing all 4 genes and ≥3 genes, respectively. The blaAmpC gene is more prevalent than other genes, and this is probably due to the prevalence or rapid transfer of this beta-lactamase. However, more studies should be performed in a comparative way to isolate and identify other antibiotic-resistant bacterial strains associated with other hospitals.

1. Introduction

Microbial infections, heart diseases, and cancers are the main health-threatening diseases worldwide [1, 2]. Antibiotic resistance is a threat in many hospitals, and mortality and death due to infections in patients are increasing dramatically [3]. Antimicrobial resistance often occurs as a result of the treatment of infectious diseases by misuse of antibiotics and is a global problem that affects the environment, human and animal health, as well as agriculture and the economy [4, 5]. Bacterial resistance to antibiotics can be either innate or acquired [6]. Meanwhile, antibiotic resistance has become a global problem among the clinical strains of *Acinetobacter, Klebsiella, Pseudomonas aeruginosa, Proteus, Escherichia coli,* and *Enterobacter* species, and becomes a concern when this resistance can be transmitted through motile genetic elements (MGE) [7]. Among these strains, *Acinetobacter baumannii* (Figure 1) plays an important role due to its ability to acquire resistance genes and cause a wide range of nosocomial infections, including bacteremia, secondary meningitis and urinary tract infections [8]. *Acinetobacter* is gram-negative coccobacilli, catalase-positive, oxidase-negative, non-fermentative and aerobic that is widely distributed in the hospital with difficulty to treat pathogens particularly in Intensive Care Units (ICUs) [9].

A. baumannii has a great potential for the rapid development of antibiotic resistance, which today has led to multidrug-resistance. This bacterium is one of the six most

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important multidrug-resistant (MDR) microorganisms in hospitals around the world. The prevalence of Pandrug –Resistant (PDR) *A. baumannii* has been established in Asian and Middle Eastern hospitals, and various types of carbapenemases have been reported [10]. Currently, several *A. baumannii* strains have become resistant to all available antimicrobial agents with the ability to transmit antibiotic resistance genes through chromosomes, plasmids, and transposons. Dependent on the type of bacteria, resistance mechanisms also take place in different ways.

The most important mechanisms of antibiotic resistance in *A. baumannii* isolates such as target site change, overexpression of genes associated with efflux pumps, decreased permeability, penicillin-binding proteins (PBP), and enzymatic inactivation of drugs such as beta-lactamases. Inactivation of beta-lactam antibiotics by beta-lactamase enzymes is one of the most important resistance mechanisms in *A. baumannii* [11]. These enzymes inactivate beta-lactam antibiotics by hydrolyzing the beta-lactam ring, making a complicated antimicrobial therapy. Beta-lactamase enzymes are grouped into four molecular classes A-D. The updated classification of these enzymes involves metallo-β-lactamases as group 3; classes A and D (group 2) broad-spectrum, extended-spectrum β-lactamases and serine carbapenemases; class C (group 1) cephalosporinases [12]. Treatment of *Acinetobacter* infections is often difficult because of their resistance to multiple antibiotics [13-15].

Carbapenems are currently used as the common drugs for the treatment of MDR *Acinetobacter* infections. However, by the emergence and increase of strains with resistance to carbapenems, treatment of *Acinetobacter* infections is considered as an important health problem in many countries [16]. The issue of isolating the resistant *A. baumannii* strains and identifying their resistance mechanisms seems to be very important, and accurate and reliable results in this field are important to apply appropriate treatment strategies and prevent the spread of infection in hospitals as nosocomial infections.

Due to the lack of knowledge about the prevalence of *Acinetobacter* infections in Baghdad hospitals in Iraq and determination of the profile of the antibiotic resistance and genotype of antibiotic resistance in strains isolated from these hospitals, this study was performed.

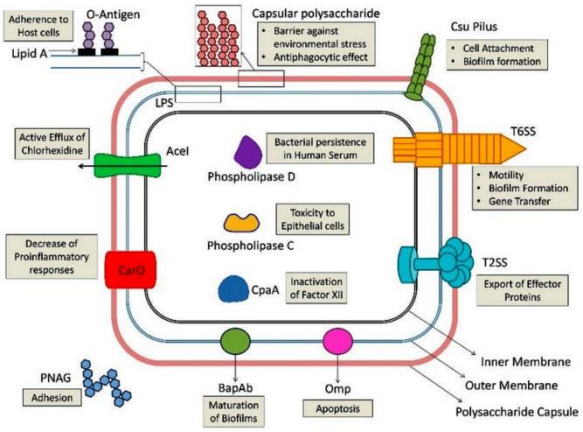

Fig. 1. A schematic image of virulence factors of *A. baumannii*. Copyright under the terms and conditions of the Creative Commons Attribution (CC BY) license (**http://creativecommons.org/licenses/by/4.0/**)[8].

2. Materials and methods

2.1. Collection and identification of bacterial strains

In a cross-sectional descriptive study, from January to the end of June 2019, 200 clinical specimens of patients admitted to the ICU of Baghdad hospitals were collected. Samples were collected from blood, throat, urine, catheter, wound and lung. After collection, the samples were transferred to the laboratory and cultured on Blood agar and MacConkey agar (Merck Germany) and then incubated at 37 ° C for 24 hours. Standard biochemical tests and the VITEK®2 system were used to identify the bacterial strains [17].

2.2. Antibiotic susceptibility test

Antimicrobial susceptibility test was determined according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [18]. The antibiotic discs used in this study were included cefotaxime (30 µg), levofloxacin (5 µg), ceftazidime (10 µg), amoxicillin / clavulanic acid (10-20 µg) and

ceftriaxone (30g), imipenem (10 µg).), meropenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), cefixime (5 µg) and cotrimoxazole (1.25 / 23.75 µg). To evaluate the antibiotic susceptibility test, a certain volume of the suspension of bacterial strains with McFarland standard (1.5×10^8 CFU / ml) was inoculated and spread on Müller-Hinton agar medium (Merck Germany). Then, each of the disks was placed on the plate at specific intervals. Finally, after incubation for 24 hours, the inhibition zone diameter was measured and the results were interpreted as sensitive, intermediate and resistant forms [19].

2.3. The presence of blaAmpC, blaTEM, blaVIM, and blaSHV genes

The conventional boiling method was used for fast and easy extraction of bacterial DNA. Briefly, several pure colonies were removed from the 24-hour culture of bacterial strains and inoculated into 100 µl of sterile distilled water. The microtubes were then kept at 100°C for 15 minutes. After a time, the microtubes were placed in the freezer for 5 minutes for temperature shock and cell wall lysis. The microbial suspension was then centrifuged at 12,000 rpm for 10 minutes, and the resulting supernatant was added to the new microtubes as a DNA template [20].

Polymerase chain reaction (PCR) method was used to evaluate the presence of blaAmpC, blaTEM, blaVIM and blaSHV beta-lactamase genes. Table 1 shows the sequence of the primers, the annealing temperature, and the target genes. The materials required for the PCR method were as follows: 9 µl of Master Mix, 1 µl of each of the forward and reverse primers (10 pmol/uL), 2 µl of template DNA, and 12 µl of deionized water. Except for annealing temperature, the reaction temperature of the PCR for all beta-lactamase genes was the same. The PCR cycling conditions were as follows: 94 ° C for 5 min, followed by 35 cycles at 94 ° C for 45 s, and a final 5-min extension step at 72 ° C. Finally, the PCR products were analyzed by electrophoresis in 1 % agarose gel. A negative control sample was used in each PCR reaction containing all components except the DNA template which was replaced with distilled water. A 100 Bp marker was used as a standard.

2.4. Statistical analysis

The results were analyzed using the 26th version of the software (IBM Co., SPSS Statistics) SPSS and Chi-square and Fisher's tests. The significance limit was set at $p < 0.05$.

3. Results

The results of biochemical tests and VITEK®2 system showed that out of 200 samples collected from patients admitted to ICU, *Pseudomonas* (24.3%), *Staphylococcus* (21.2%), *A. baumannii* (19%), *E. coli* (18.1%) *Klebsiella pneumoniae* (13.1%) and *Enterobacter* (4.3%) had the highest number of samples, respectively. Out of 38 strains of *A. baumannii*, 21 samples (55.2%) were related to male patients with a mean age of 38 years and 17 samples (44.8%) were related to female patients with a mean age of 36 years. Among the *A. baumannii* strains, 5 (13.1%) samples of blood, 7 (18.4%) from throat, 7 (18.7%) from urine, and 3 (7.8%) from lung samples, 5 (13.1%) from catheter and 11 (28.9%) from wound samples were obtained ($p < 0.05$) (Table 2). The results of antibiotic susceptibility testing are also shown in Table 3. As can be seen, the highest antibiotic resistance among *A. baumannii* isolates was related to amoxicillin / clavulanic acid (92.1%), imipenem (86.8%) and ceftriaxone (86.8), respectively.

The highest antibiotic susceptibility was related to tobramycin (31.5%), co-tri-moxazol (26.3%), and ciprofloxacin (26.3%). In this study, the tobramycin antibiotic showed better activity than other antibiotics against MDR *A. baumannii*. However, other antimicrobial agents such as amoxicillin / clavulanic acid, imipenem and ceftriaxone showed high rates of the resistance in the present study. The results of antibiogram revealed that the level of resistance to carbapenems and cephalosporins is higher compared to other classes of antibiotics.

Table 1. Product length and primers sequence used for PCR reaction.

Gene	Primer	Sequence	TM (oC)	Size (bp)	References
blaAmpC	forward reverse	5´- ACTTACTTCAACTCGCGACG -3´ 5´- TAAACACCACATATGTTCCG-3´	50	663	[21]
blaVIM	forward reverse	5´- GATGGTGTTTGGTCGCATA-3´ 5´- CGAATGCGCAGCACCAG-3´	51	390	[22]
blaTEM	forward reverse	5´- AGGAAGAGTATGATTCAACA -3´ 5´- CTCGTCGTTTGGTATGGC -3´	52	535	[23]
blaSHV	forward reverse	5´-AGCCGCTTGAGCAAATTAAAC-3´ 5´-ATCCCGCAGATAAATCACCAC-3´	53	713	[24]

Table 2. Collected samples from blood, throat, urine, catheter, wound, and lung with related frequency of their beta-lactamase genes.

Gene	frequency	catheter n = 5	wound n = 11	blood n = 5	urine n = 7	throat n = 7	lung n = 3
AmpC	38	5	11	5	7	7	3
VIM	30	4	9	2	5	7	3
TEM	28	3	7	3	7	6	2
SHV	21	4	8	2	1	5	1

Table 3. Antibiotic resistance profile for *A. baumannii* strains.

Antibiotic classes	Antibiotics	Resistant	Intermediate	Sensitive
Carbapenems	Imipenem (10µg)	33 (86.8 %)	0 (0 %)	5 (13.1 %)
	Meropenem (10µg)	31 (81.5 %)	0 (0 %)	7 (18.4.2 %)
Cephalosporins	Ceftazidime (30 µg)	31 (81.5 %)	0 (0 %)	0 (0 %)
	Ceftriaxone (30 µg)	33 (86.8 %)	1 (2.6 %)	4 (10.5 %)
	Cefixime (5 µg)	26 (68.4 %)	3 (7.8 %)	9 (23.6 %)
Quinolones	Ciprofloxacin (5 µg)	27 (71 %)	1 (2.6 %)	10 (26.3 %)
	Levofloxacin (10µg)	29 (76.3 %)	5 (13.1 %)	4 (10.2 %)
Aminoglycosides	Gentamicin (10 µg)	31 (81.5 %)	0 (0 %)	7 (18.4 %)
	Tobramycin (10 µg)	23 (60.5 %)	3 (7.8 %)	12 (31.5 %)
Penicillins	Amoxicillin / Clavulanic acid (20/10 µg)	35 (92.1 %)	1 (2.6 %)	2 (5.2 %)
sulfonamides	Co-tri-moxazol (1.25/23.75 µg)	26 (68.4 %)	2 (5.2 %)	10 (26.3 %)

PCR results for blaAmpC , blaVIM, blaSHV, and blaTEM genes in 38 strains of *A. baumannii* showed that the blaAmpC gene was more common than all the other genes and was detected in 100% (38) of the isolates. The frequencies of blaVIM, blaTEM and blaSHV genes were determined in 78.9% (30), 73.6% (28), and 55.2% (21) of *A. baumannii* strains, respectively (Figure 2). The presence of blaAmpC , blaVIM, blaSHV, and blaTEM genes indicates the possible role of these genes in the antibiotic resistance of *A. baumannii* isolates. The prevalence of genes is shown in Fig. 2. According to PCR results (Figure 2a-d), it was found that the studied beta-lactamase genes were involved in antibiotic resistance. Molecular analysis also showed that about 18.4% of the isolates expressed all 4 genes. More than 53.5% of isolates had at least 3 genes. The relationship between the source of isolates and the presence of genes was not statistically significant (P <0.05).

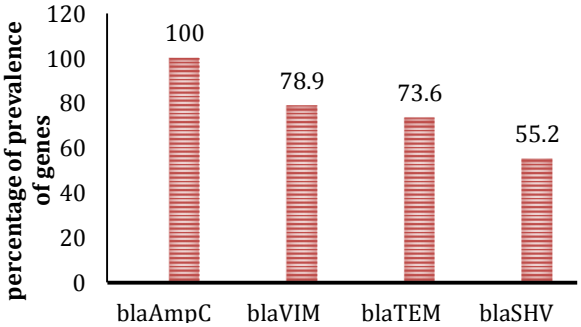

Fig. 2. Percentage of detected genes in some *A.baumannii* strains.

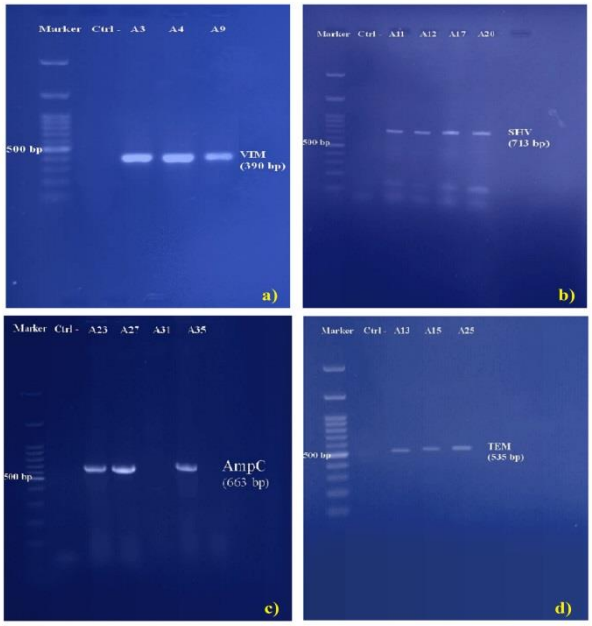

Fig. 2. Detection of *bla*$_{VIM}$ (a), *blaSHV* (b), *blaAmpC* (c), and *blaTEM* (d) genes in some *A. baumannii* strains.

4. Discussion

A. baumannii is an emerging and opportunistic hospital pathogen responsible for nosocomial infections. The development of multidrug resistance in *A. baumannii* is a growing concern. *A. baumannii* plays an important role in nosocomial infections, especially in ICU patients [8]. This study was performed to determine the antibiotic resistance profile and evaluate the presence of beta-lactamase genes among *Acinetobacter* spp. strains isolated from clinical specimens. All isolates in this study were resistant to many antibiotics. Our results showed that the resistance rate to cephalosporins and carbapenems is high. The detection of MDR strains of *A. baumannii* has increased dramatically in Iraq in recent years. Numerous studies have been conducted in this regard in Iraq.

In the study of Shali *et al.* [25] the resistance to imipenem was 57.1%, which was lower than the findings of the present study, however, the resistance to gentamicin, ceftazidime and amoxicillin / clavulanic acid was similar to our results [25]. In another report, the resistance of *A. baumannii* strains to cefotaxime (94%), ceftriaxone (83%), amoxicillin + clavulanic acid (85%), ceftazidime (80 %) and ciprofloxacin (73%) were consistent with our observations [26]. In the report of Aziz *et al.* [27] the resistance to the aminoglycoside antibiotics of gentamicin and tobramycin among *A. baumannii* strains was similar to the reported finding [27].

In other countries, many studies have been performed to evaluate antibiotic resistance among *A. baumannii* strains. A study by Lin Yin et al. [23] in China and a study by Lowings *et al.* [28] in South Africa reported 100% resistance to cefotaxime. Also, in the study of Al-Agamy and coworkers in Egypt [29] the resistance of the isolates to the two antibiotics cefotaxime and ceftazidime was 100% [29]. In the report of F. Mushi *et al.*, the resistance to ceftazidime was estimated to be 90% [30]. All the above reports are completely consistent with our results of the present study and indicate that the resistance of *Acinetobacte baumannii* strains to cephalosporins is very high. In the study of Chang *et al.* in China [21] and also in the study of Al-Agamy *et al.* in Egypt [29], the prevalence of AmpC beta-lactamase was higher than other genes and was detected in 97.1% and 100% of Carbapenem-resistant *A. baumannii* strains, respectively. Also, according to studies conducted by Hujer *et al.* [31] and Lin Yin *et al.* [23], this gene was the most common among the studied genes and was reported in 99% and 100% of MDR isolates, respectively. All the results of the above studies are consistent with our results. The incidence of the blaVIM gene in the present study was also high and was detected in 78.9% of *A. baumannii* isolates. The VIM beta-lactamase gene belongs to the group of beta-lactamases that make carbapenem-resistant. Although this gene is less common than previous studies, it is the most common metallobetalactamase gene. Aziz *et al.* [27] reported the prevalence of this gene among *A. baumannii* strains isolated from clinical samples of Najaf and Baghdad hospitals in Iraq, which did not correspond to our findings [27]. The frequency of this gene was different in other countries as well. In the studies of Fallah *et al.* the prevalence of the mentioned gene was reported 44 17 % and 32.6%, respectively [32].

In some studies, were related that the blaVIM gene was not detected in any of the

resistant isolates of *A. baumannii* [21, 23]. In Satir *et al*. [31] study, the prevalence of this gene in carbapenem-resistant isolates was estimated to be 67.56%, which in all of the above cases, the spread of this gene in *A. baumannii* strains is less than the present study. Probably the reason for the rapid spread of this gene in the isolates of *A. baumannii* is the placement of this gene on the plasmid and as a result of transfer through the plasmid to other susceptible bacteria and their conversion into a resistant form [31]. PCR results in the present study showed that 55.2% of *A. baumannii* strains have the blaSHV gene. SHV beta-lactamase belongs to class A beta-lactamases called broad-spectrum (ESBL). This gene is located on a plasmid or chromosome and is responsible for resistance to broad-spectrum cephalosporins. The blaSHV gene is commonly found in Enterobacteriaceae, but there are reports from around the world showing an increase in *A. baumannii* bacteria. Al-Hasnawy *et al*. [26] have shown that 15.3% and 7.6% of *A. baumannii* isolates were able to carry bla-SHV and bla-TEM-2 genes, respectively [26]. Hujer *et al*. [31], Lin Yin *et al*. [23] reported that the prevalence of this gene in *A. baumannii* isolates was 1% and 0%, respectively. The prevalence of SHV gene in Agamy *et al*.'s study among carbapenem-insensitive *A. baumannii* strains was determined to be 0% [29]. According to these reports, the prevalence of this gene in all of the above studies is much lower than the present study. According to the reports, it seems that the prevalence of SHV gene in *A. baumannii* isolates has increased over time. TEM beta-lactamase is another antibiotic-resistant gene that is located on the plasmid and is resistant to broad-spectrum cephalosporins. PCR results in the present study indicate the presence of this gene in 73.6% of the isolates. In the report of Al-kadmy *et al*. [33], the prevalence of blaTEM gene among *A. baumannii* strains isolated from clinical samples of Baghdad hospitals was estimated to be about 20% [33].

5. Conclusion

Increasing resistance to carbapenems and cephalosporins antibiotics is a major challenge in the treatment of *A. baumannii* infections. In this investigation, PCR results demonstrated that all *A. baumannii* isolates had beta-lactamase genes, also the highest prevalence of beta-lactamase genes was related to blaAmpC, blaVIM, blaTEM and blaSHV genes, respectively. AmpC belongs to group C beta-lactamases and is also called cephalosporinase. The blaAmpC genes are located on the plasmid or chromosome of *A. baumannii* strains. In addition, it seems that the blaAmpC gene is more prevalent than other genes, and this is probably due to the prevalence or rapid transfer of this beta-lactamase gene. Finally, future studies should be performed in a comparative way to isolate and identify other resistant strains relate to other hospitals.

Abbreviation

PCR: polymerase chain reaction
MDR: multidrug-resistant
PDR: pandrug–resistant
PBP: penicillin-binding proteins
MGE: motile genetic elements
ICUs: Intensive Care Units
CLSI: Clinical and Laboratory Standards Institute

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Conflict of interest

The authors declare no conflict of interest.

Consent for publications

The authors read and proved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article

Authors' Contribution

Study concept and design: Z. K. A. A- K and Q. H. A. A. Data acquisition: Z. K. A. A- K and Q. H. A. A. . Data analysis and interpretation: Z. K. A. A- K and Q. H. A. A.

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Ethics approval and consent to participate

The study does not need ethical approval.

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