

Molecular Identification of Biocide Resistant Quaternary Ammonium Compound (QAC) Genes in Multi-Drug Resistant *Acinetobacter baumannii* Isolated from ICU Patients in Erbil City

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Abstract: Multidrug resistant *Acinetobacter baumannii* (MDR-AB) is recognized as one of the most important nosocomial pathogens in Intensive Care Unit (ICU) patients. Disinfectants are frequently used in hospitals to prevent transmission of MDR-AB-related infections. However, the excessive use of disinfectants may impose selective pressure on MDR-AB strains and lead to wide spread of biocide Quaternary Ammonium Compound (QAC) resistance genes. This study aimed to investigate 40 MDR-AB isolates collected from ICU patients for the distribution of QAC genes (*qacE* and *qacAE1*) by Polymerase Chain Reaction (PCR) method and the susceptibility towards benzalkonium (BAC) and Didecyldimethylammonium chloride (DDAC) by Agar Well Diffusion method. Results showed that 32 (80%) and 30 (75%) isolates harbored *qacE* and *qacAE1* genes, respectively. All isolates showed high susceptibility against tested biocides, in which the mean of growth inhibition zone for each of BAC and DDAC were 24mm and 23 mm, respectively. In conclusion, this study confirms high frequency of QAC genes in MDR-AB isolates. Moreover, efficient microbiological efficacy of these biocide agents was observed as expected according to the manufacture's standards guideline.

Keywords: *Acinetobacter Baumannii*, Biocide Resistance Genes, Multidrug Resistance

1. Introduction

Acinetobacter baumannii is an important opportunistic Gram-negative pathogen that has been shown to cause life-threatening nosocomial infections including ventilator-associated pneumonia, wound infections and bloodstream infections especially in severely unwell patients in intensive care units with a mortality rate of 20-60% (Xiao et al., 2017).

It has a considerable potential to colonize and persists for a prolonged period on inanimate hospital surfaces under disinfected environment. Moreover, due to its intrinsic multiple drug resistance this pathogen can easily cause nosocomial outbreaks (Sousa et al., 2021). The majority of hospital acquired *A. baumannii* isolates are resistant to different classes of antibiotic including penicillins, cephalosporins, carbapenems, aminoglycosides and fluoroquinolones. Multidrug-resistant (MDR) *A. baumannii* isolates are a serious problem in healthcare settings worldwide (Safari et al., 2013). Therefore, management of MDR-AB infections is considered as a great challenge for hospitalized patients and clinicians (Russo et al., 2022). The major sources of cross-transmission of MDR-AB infections are direct or indirect contact through hands of colonized patient and healthcare workers, or through contaminated surfaces and previous room occupancy by infected patients with MDR-AB.

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The major sources of cross-transmission of MDR-AB infections are direct or indirect contact through hands of colonized patient and healthcare workers, or through contaminated surfaces and previous room occupancy by infected patients with MDR-AB. Recent studies have shown that adherence to infection control interventions have important role in reducing the transmission of this pathogen in the hospital environment, nosocomial infection rates and consequently prevents the occurrence of MDR-AB outbreaks. For instance, cleaning and disinfection of surfaces, hand hygiene compliance, surveillance of nosocomial infections and regular detection of environmental microbial contamination, are among the most important intervention for preventing transmission of MDR-AB pathogens in hospital (Byun et al., 2021).

Disinfectants are frequently used in hospitals to prevent transmission of MDR-AB-related infections. However, the excessive use of disinfectants over the recommended frequency and concentration may impose selective pressure on MDR-AB strains. Consequently, this may lead to emergence and wide spread of biocide resistance genes (BRG) (Vijayakumar and Sandle, 2019).

Recent studies confirmed many BRG in MDR pathogens, such as quaternary ammonium compound (QAC) resistance genes which are mobile genetic-borne elements including *qacE* and *qacΔE1* genes (Gomaa et al., 2017). *A. baumannii* possesses multidrug transporter efflux systems, expression of efflux systems involving *qac* genes (*qacE* and *qacΔE1*) is one of the biocide resistance mechanisms which are propagated in gram-negative bacteria worldwide (Chang et al., 2007). Basically due to high spread of plasmid-mediated class 1 integrons, which commonly include *qacΔE1* (Kazama et al., 1998). The ability of *qac* genes to offer resistance to antibiotics remains unspecified. Nevertheless, a close association between resistance to antibiotics and biocides can be explained by the fact that the class 1 integrons (mobile genetic elements) hosts a variety of antibiotic resistance genes (Zhao et al., 2012). Owing to these facts, there is a rational concern that the inadequate use of biocides could select for antibiotic-resistant bacteria in Gram-negative bacteria (Hegstad et al., 2010).

Although many reports focus on the increasing resistance of *A. baumannii* strains to antibiotics, few studies have investigated the susceptibility of *A. baumannii* to biocides (Kawamura-Sato et al., 2010); also, there is little information available that weighs the risks of antibiotic resistance induced by increased resistance to biocides in Iraq. Understanding the susceptibility of *A. baumannii* to disinfectants and its correlation with antibiotic resistance will undoubtedly contribute to the control of this microorganism in hospitals.

The aim of this study was to investigate MDR-AB isolates collected from ICU patients for distribution of QAC genes (*qacE* and *qacΔE1*) and the susceptibility towards QAC biocide agents including benzalkonium (BAC) and Didecyldimethylammonium chloride (DDAC).

2. Materials and Methods

2.1 Clinical Sample Collection & Microbial Identification

In our study, a total of 150 non-repetitive clinical specimens including endotracheal secretions, wound swabs, sputum, blood, and urine samples were collected from microbiology laboratories of different hospitals, in Erbil city, Iraq between Septembers to December 2021. Isolation and identification of *A. baumannii* were carried out according to standard microbiological techniques, as follows: Patients samples were cultured on blood agar (Oxoid Ltd., Basingstoke, UK), MacConkey agar (Oxoid Ltd., Basingstoke, UK), and incubated at 37°C under aerobic conditions for 18–24 h. Following overnight

incubation, *A. baumannii* isolates were identified according to culture characteristics such as identification of Gram-negative pleomorphic bacilli, catalase positive, non-motile (A'shimi et al., 2019). Moreover, the identification of *A. baumannii* isolates was confirmed using an automated bacterial identification system VITEK® 2 compact system (BioMérieux). This system utilizes a new fluorescence-based technology for bacterium detection and antimicrobial susceptibility testing in accordance with Clinical and Laboratory Standard Institute (CLSI) recommendations (Park et al., 2009). In addition, polymerase chain reaction (PCR) was employed to amplify the biocide resistance genes *qacE* and *qacΔE1* gene and *blaOXA-51-like* gene which is intrinsic to this species. The size of PCR product that amplifies the open reading frame of the *blaOXA-51-like* carbapenemase gene was 353 bp (Nigro and Hall, 2018), as shown in Table 1.

Table 1. Primer sequences, product sizes, and annealing temperatures for biocide resistance-encoding genes in clinical isolates of *A. baumannii*

Targeted genes	Primer sequences (5'-3')	Product size (bp)	Annealing Temperature (°C)	References
<i>Bla_{OXA-51}</i> gene	F:TAATGCTTTGATCGGCCTTG	353bp	44°C	(Turton et al., 2006)
	R:TGGATTGCACTTCATCTTGG			
<i>qacE</i> gene	F:CGCAATAGTTGGCGAAGTAA TCG	250bp	50°C	This study
	R:CCCATACTTACAAAGCCCC			
<i>qacΔE1</i> gene	F:GCGAGGGCTTTACTAAGCTTG C	259bp	52°C	This study
	R:CGCAGCGACTTCCACGATGG			

2.2 Antimicrobial Susceptibility Testing (AST)

Antibiotic sensitivity testing was performed by using VITEK® 2 system using AST-GN30, according to manufacturer instructions by using following antimicrobial agents; Ticarcillin, Piperacillin, Gentamicin, Tobramycin, Amikacin, Netilmicin, Piperacillin/Tazobactam, Cefotaxime, Ceftriaxone, Ceftazidime, Cefepime, Imipenem, Meropenem, Ciprofloxacin, Levofloxacin, Tetracycline, Tigecycline, Trimethoprim/sulfamethoxazole, Ampicillin/Sulbactam, Colistin, minocycline, and Ticarcillin/Clavulanic acid. Results of Vitek susceptibility testing were obtained as MIC values and shown as susceptible (S), or resistant (R), according to the updated CLSI criteria, MIC breakpoints (CLSI, 2020). An *A. baumannii* isolate is defined as multiple drug resistance *Acinetobacter baumannii* (MDRAB) when it is resistant to more than one antimicrobial agent in three or more antimicrobial classes (Magiorakos et al., 2012).

2.3 PCR Amplification

The PCR technique was performed to detect the *qacE* and *qacΔE1* genes among *A. baumannii* isolates. All primer sequences are described in Table 1. Total genomic DNA of the strains were extracted using

the DNeasy tissue kit (Qiagen, Germany), according to the manufacturer's instructions. All DNA extracts were stored at -20 °C before PCR amplification.

PCR amplification was performed as described in (Baban, 2020). In brief, Initial denaturation at 95°C for 5 min, denaturation at 95°C for 30s, annealing at 44°C–52°C for 30s., followed by elongation at 72°C for 30s., and final elongation step at 72°C for 5 min to complete the extension of the primers. Finally, the PCR product was kept at 4°C until analysis. The list of oligonucleotide sequences used in this study and the PCR amplification conditions, particularly annealing temperature and elongation time are shown in Table 1.

The PCR amplification reaction was prepared in a total volume of 20 µL containing 10 µM of each of forward and reverse primers (Eurofins, Germany), 1.5 mM of MgCl₂, 10 µM of dNTPs mixture, 10X GoTaq® Green buffer, Taq DNA polymerase, and the reaction mixture was filled up to final volume with Nuclease-free water. Following amplification, PCR products were separated by electrophoresis with 1% (w/v) agarose gel in TBE buffer (40 mM Tris, 1 mM EDTA and 0.1% (v/v) boric acid). The agarose gel was stained with ethidium bromide (10 µg/ml), and PCR products were visualized on the ultra-violet transilluminator.

2.4 Biocide Susceptibility

Biocide solutions containing benzalkonium (BZK) (Big extra), and Didecyldimethylammonium chloride (DDAC) (Surfanios) were tested by using agar well diffusion method. A standard bacterial concentration of McFarland Standard 0.5 (1.5×10^8 CFU/mL) was used. In brief, 50 µl of bacterial suspension was streaked on Mueller-Hinton agar plates. Then, a well with a diameter of 5 mm was punched using a sterile cork borer. Subsequently, the wells were impregnated with 50µl of each biocide prepared solution. Each biocide solution was prepared according to manufacturer's instructions. During the incubation period at 37°C for 18–24 h., the tested material diffuses from the hole into the agar medium seeded with the test microorganism. Following incubation, the clear zone of inhibition around the biocide agent was measured in millimeter. The experiments were performed in triplicates (Dhayalan et al., 2018)

2.5 Statistical Analysis

The data were statistically analyzed by SPSS version 15 (SPSS Inc., Chicago, IL, USA). The Chi-square test was provided to compare categorical data. $P < 0.05$ was considered statistically significant.

2.6 Ethical Consideration

This study was achieved according to the ethical committing at Hawler Medical University and all persons had given their acceptance to share in the study.

3. Results

3.1 Isolation and Identification of *A. baumannii* isolates

In this study, a total of forty (26.6%) non-repetitive *A. baumannii* strains were identified from 150 clinical specimens collected from ICU patients. The most frequent source of isolation of *A. baumannii* were detected in 12 endotracheal secretions (30.0%), 11 surgical-site wound infection (27.5%), 8 sputum (20.0%), 7 blood (17.5%) and 3 urine (5.0%), as described in Figure 2. Genotypically, all *A.*

baumannii isolates were confirmed by PCR amplification for the presence of β -lactamase gene *bla*_{OXA-51-like} which is originally intrinsic to *A. baumannii*, as described in Figure 1.

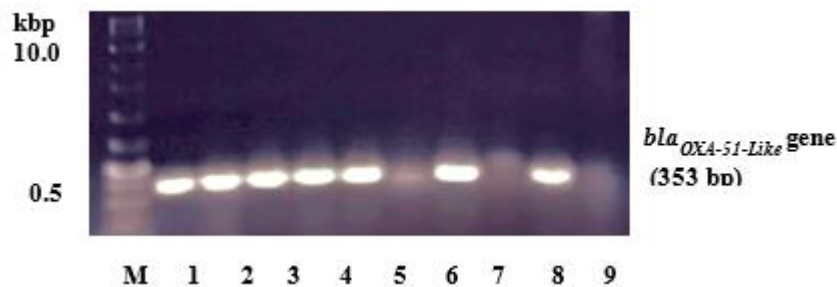


Figure 1: A presentation of PCR screening analysis of *bla*_{OXA-51-like} genes (353bp) detection in *A. baumannii* isolates. Lane M, DNA ladder (10kbp), lanes (1,2,3,4,5,7,9) represent *A. baumannii* isolates.

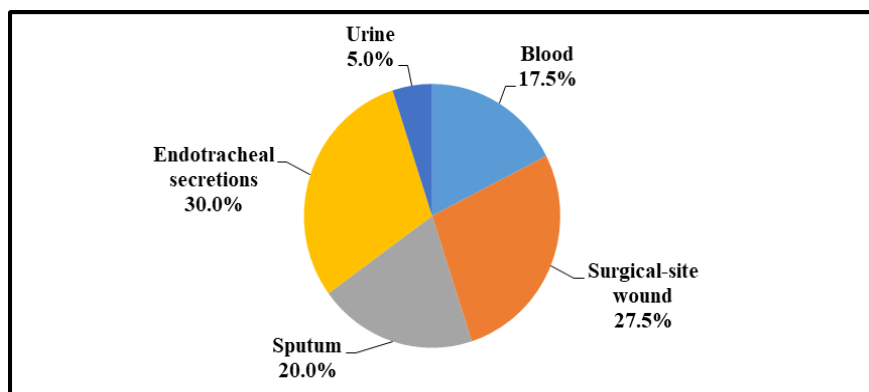


Figure 2: Percentage of *A. baumannii* in relation to the type of clinical specimens

3.2 Antimicrobial Susceptibility Profile of *A. baumannii* isolates

The antimicrobial susceptibility patterns of all *A. baumannii* isolates against 22 antimicrobial agents from 11 antimicrobial classes are presented in Table 2. The most effective antimicrobial group against *A. baumannii* isolates was Polymixins (Colistin) (62.5%). The results revealed that all isolates were 100% resistant against Penicillins (Ticarcillin and Piperacillin), aminoglycosides (Amikacin), carbapenems (Meropenem), antipseudomonal fluoroquinolones (Ciprofloxacin and Levofloxacin), extended-spectrum cephalosporins (Cefotaxime, Ceftriaxone, Cefepime), and Ampicillin-sulbactam. Moreover, the isolates were varied in their resistance to the following antimicrobial agents including imipenem, piperacillin-tazobactam and ceftazidime (97.5%), followed by tobramycin, Netlimicin, Ticarcillin-calulvalic acid (95.0%), Tetracycline (92.5%), Gentamicin (90.0%), Tigecycline (85%), Trimethoprim-sulphamethoxazole (70%), and minocycline (65%).

3.3 Presence of Biocide Resistant Genes in *A. baumannii* isolates

Among the 40 isolates tested, the biocide resistant genes *qacE* and *qacΔE1* were detected in 32 (80%) and 30 (75%) isolates, respectively. This indicates that these genes were dominant in MDR *A. baumannii* isolates. Furthermore, all isolates showed susceptibility against hospital biocides containing quaternary ammonium core compounds, in which the mean of growth inhibition zone for each of BAC and DDAC were 24mm and 23 mm, respectively, as shown in Figure 3.

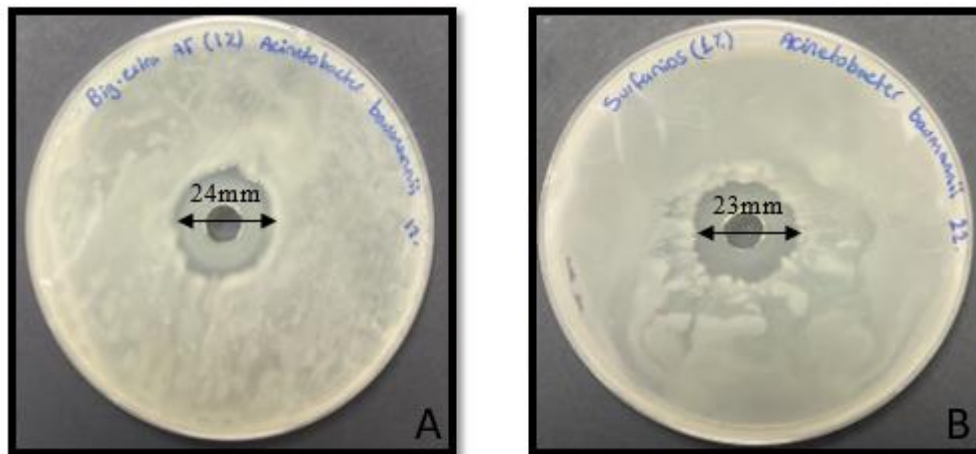


Figure 3: Antibacterial activity of biocides (A) BAC and (B) DDAC against MDR *A. baumannii* by Agar well diffusion method.

Table 2: Prevalence of antimicrobial resistant *Acinetobacter baumannii* isolates against antimicrobial agents in eleven antimicrobial classes

Antibiotic categories	Antimicrobial agent	Sensitive %	Resistance %
Penicillins	Ticarcillin	0(0.0%)	40 (100%)
	Piperacillin	0(0.0%)	40 (100%)
Aminoglycosides	Gentamicin	4(10.0%)	36(90.0%)
	Tobramycin	2(5.0%)	38(95.0%)
	Amikacin	0(0.0%)	40(100%)
	Netilmicin	2(5.0%)	38(95.0%)
Antipseudomonal carbapenems	Imipenem	1(2.5%)	39(97.5%)
	Meropenem	0(0.0%)	40(100%)
Antipseudomonal fluoroquinolones	Ciprofloxacin	0(0.0%)	40(100%)
	Levofloxacin	0(0.0%)	40(100%)
Antipseudomonal penicillins+ β -lactamase inhibitors	Piperacillin-tazobactam	1(2.5%)	39(97.5%)
	Ticarcillin-clavulanic acid	2(5.0%)	38(95.0%)
Extended-spectrum cephalosporins	Cefotaxime	0(0.0%)	40(100%)
	Ceftriaxone	0(0.0%)	40(100%)
	Ceftazidime	1 (2.5%)	39(97.5%)
	Cefepime	0(0.0%)	40(100%)

Folate pathway inhibitors	Trimethoprim-sulphamethoxazole	12(30.0%)	28(70.0%)
Penicillins+ β -lactamase inhibitors	Ampicillin-sulbactam	0(0.0%)	40(100%)
Polymyxins	Colistin	25(62.5%)	15(37.5%)
Tetracyclines	Tetracycline	3(7.5%)	37(92.5%)
	Minocycline	14(35.0%)	26(65.0%)
Glycylcyclines	Tigecycline	6(15.0%)	34(85.0%)

4. Discussion

Multidrug-resistant *Acinetobacter baumannii* is recognized as one of the most difficult antimicrobial-resistant gram-negative opportunistic pathogen to control and treat. Rising prevalence of MDR-AB has posed a major challenge in patient care and infection control unit in healthcare system worldwide. Prolonged length of hospital stays of postoperative patients, receiving mechanical ventilation in intensive care unit, transmission of this opportunistic pathogen by the hand of healthcare staff and frequent exposure of hospitalized patients to antimicrobial agents are the most important risk factors for colonization or infection with MDR *A. baumannii*. Rapid emergence and unfortunate acquisition of MDR-AB that cause pneumonia, wound infection, septicemia particularly among ICU patients in the hospital could be prevented and reduce the risk of transmission of life-threatening infections if implementing, practicing and monitoring the infection control standard and hand hygiene are performed regularly (Ghafur et al., 2014) and (Çelik, 2014).

Hospital biocide agents are widely used to prevent the dissemination of MDR pathogens in the environment. They are used for the purpose of disinfection of inanimate objects, various surfaces of medical devices such as ventilators and catheters to clean from microbial contamination. Recent studies have reported both the present of plasmid-mediated biocide resistance genes (QAC) such as *qacE* and *qac Δ E1* and reduced tolerance to biocide agents. Other studies have investigated that co-carriage of both biocide resistance genes and antibiotic resistance genes on plasmids could contribute to the development of increased resistance in hospitalized pathogens. This study was conducted to evaluate the inhibitory effect of frequently used biocide agents in accordance with the manufacturer's recommendations to clinical isolates of MDR-AB. According to results of this study, 80% and 75% of MDR-AB isolates harbored *qacE* and *qac Δ E1* genes, respectively. In a study conducted in Iran, the prevalence of genes *qac Δ E1* and *qacE* among MDR *A. baumannii* isolates were 91.0% and 4.0%, respectively (Shirmohammadlou et al., n.d.). another study by (A'shimi et al., 2019) noted the prevalence of *qacE* and *qac Δ E1* 28.0% - 63.0, respectively. Furthermore, all biocide agents with active ingredients BAC and DDAC tested against MDR-AB isolates displayed similar growth inhibition zone phenotype. These results confirm efficient microbiological efficacy of these biocide agents as expected in the manufacture's standards guideline (Chavignon et al., 2021).

5. Conclusion

Alarmingly, findings of this study demonstrate that MDR-AB isolates have high biocide resistance genes in MDR-AB isolates combined with its intrinsic antimicrobial resistance could pose a significant challenge to prevention and control of MDR-AB related infections. Appropriate use of infection

control interventions and regular environmental surveillance play pivotal role in reducing the distribution of QAC resistance genes among MDR-AB strains.

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