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Detection of OXA-23, OXA-48, OmpA and bla-TEM genes in carbapenem-resistance *Acinetobacter baumannii* and Biofilm formation in burn patients at Al-Anbar Government

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ABSTRACT

Background. Antibiotic-resistant bacteria represent a highly global health issue by causing high rates of morbidity and mortality in burn units, whereas biofilms significantly contribute to complications in burn patients.

Objectives. The study investigates the prevalence of (OXA-23, OXA-48, OmpA and bla-TEM) and biofilm formation of *Acinetobacter baumannii* in the Burn Unit of the Al-Anbar government. **Methods.** A total of 120 clinical swab samples from burn patient were collected from December 2023 to April 2024 from the burn units of Ramadi Teaching Hospital and Falluja Teaching Hospital for the study. The colonies were identified using conventional and Vitek methods. Colourimetric technique (MTP) and multiplex PCR were used to detect biofilms and resistance genes.

Results. Among 120 samples, 40 (33.3%) were Acinetobacter baumannii, 34 (80%) were MDR-resistant, and 6 (15%) were XDR. 90% of the isolates were resistant to Cefotaxime and Colisitin was more sensitive in all forty isolates. All 40 isolates formed significant biofilms. The percentage of positive results for OXA-23, OmpA and bla-TEM genes were 80%, 65%, and 55% of respectly. Carbapenem testing is essential to identify carbapenem-producing strains from non-producing

strains due to the high prevalence of multidrug-resistant A.baumannii with the OXA-23 and OmpA genes.

Conclusion. These findings highlight the urgent need for effective infection control measures and the development of new therapeutic strategies to manage these infections in burn patients.

Keywords: Burn, Biofilm formation, Multidrug-resistant (MDR), OXA-23, Colistin, *Acinetobacter baumannii*

INTRODUCTION

Acinetobacter baumannii is a frequent source of hospital-acquired infections in patients with burns. It is a significant opportunistic pathogen responsible for increasing mortality and morbidity rates in poor nations [1]. *A.baumannii* is ranked at the highest level on the WHO priority pathogen list, being classified as "critical." This signifies the significant importance of this hospital-acquired infection, mainly when it exhibits resistance to carbapenem, considered the final line of defense antibiotic [2]. Significant mortality has been attributed to infections induced by extensively drug-resistant (XDR) and multidrug-resistant (MDR) strains of *A.baumannii*. Furthermore, a considerable number of outbreaks have been documented on a global scale [3].

Acinetobacter can thrive under inhospitable conditions such as desiccation, antimicrobial treatments, and limited nutrition supply. The resistance mechanism is comparable to that of other Gram-negative bacteria. This process entails the utilization of hydrolyzing enzymes or modifying enzymes, efflux pumps for the expulsion of antibiotics, and less penetration of antibiotics. *A.baumannii* is characterized by its many pumps and diverse enzymes [4]. Resistant to carbapenem antibiotics. *A. baumannii* is commonly reported worldwide, with a significant prevalence of carbapenem-resistant strains in developing nations such as Korea, Pakistan, India, Chile, and Portugal, as opposed to developed countries [5].

Acinetobacter baumannii is characterized by its ability to produce biofilms, which is a significant mechanism of pathogenicity. Physicochemical parameters like as oxygen level, temperature, growing medium, surface hydrophobicity, and pH have an impact on several microbe features, including resistance genes, adhesins, capsular polysaccharides, surface appendages, and virulence genes [6]. Infections caused by *A.baumannii* can pose a significant treatment challenge

when they form a biofilm and can spread rapidly across patients, leading to difficult-to-control outbreaks. A wide variety of hospital and medical device surfaces are ideal for biofilm formation. This includes endotracheal tubes made of polystyrene, polypropylene, polytetrafluoroethylene, and glass, heart valves, prosthetic joints, ventilators, and intravascular or urinary catheters [7]. Carbapenem-inactivating enzymes, including the Class D carbapenemase (OXA enzymes), are the primary cause of resistance in *A. baumannii* globally [8]. Based on the multifactorial nature of biofilm development, various gene products, including Omp of *A. baumannii*, have been identified as significant contributors to adhesiveness and biofilm formation in *A. baumannii* [9]. The blaTEM genes also have been widely acknowledged as the most abundant genes that confer antibiotic resistance in pathogenic bacteria worldwide [10]. Apart from its capacity to generate biofilms, Acinetobacter's adeptness in acquiring and transmitting antibiotic-resistance genes confers an additional competitive edge in healthcare settings, where antibiotic utilization is consistently more significant than in other environments. Research has demonstrated that genes responsible for resistance to metals, carbapenemases, oxacillinases, metallo-beta-lactamases, and antibiotics can be horizontally transferred through different mechanisms, including outer membrane vesicles, conjugation, transformation, transfer mediated by bacteriophages, transfer mediated by nanotubes, and outer membrane transfer [11].

MATERIAL AND METHODS

Data sources

A total of 120 clinical samples from burn patients were collected from December 2023 to April 2024 from the burn units of Ramadi Teaching Hospital and Falluja Teaching Hospital for the study.

Specimens

Swab Samples are collected from different body parts using a sterile transport medium swab and then cultured on selective media (24 h at 37 °C) following bacteriology standards. Exclusion includes immunocompromised patients like diabetic patients, chemotherapy, pregnant women, etc.

Acinetobacter species identification.

Microscopic examination was performed on all samples, excluding blood, using Gram-stained smears. The samples were inoculated onto suitable culture media and incubated, and growth was

detected using standard microbiological techniques after the recommended length. Smooth, opaque colonies observed on blood agar, which matched the non-lactose fermenting colonies on MacConkey, were identified as Acinetobacter and subjected to additional analysis. The identification of species within the Acinetobacter genus was conducted using various biochemical assays, including triple sugar iron (TSI) fermentation test, oxidase, indole, motility, urease, and arginine hydrolysis [12], and by Vitek2 system (bioMérieux).

Antibiotic susceptibility test

The antibiotic susceptibility test of bacterial isolates is conducted using Kirby Bauer's Disc Diffusion method. 0.5 McFarland concentration of A.baumannii isolates were cultured on Mueller-Hinton Agar(24 h at 37 °C) [13]. 10-14 different antibiotics were used. These antibiotics include cell wall inhibitors such as Piperacillin-tazobactam, Cefepime, Aztreonam, Ceftazidime, Amoxicillin-clavulanic acid, and Imipenem.

Additionally, cell membrane inhibitors like Colistin sulfate and polymyxin-B are used, along with a protein synthesis inhibitor called Amikacin and a DNA replication inhibitor known as Ciprofloxacin. Results are interpreted according to the guidelines of CLSI-2023. Vitek2 system (bioMérieux) is also used for antibiotic susceptibility tests for Acinetobacter baumannii isolates (Figure 1).

Detection of multidrug-resistant bacteria

A recently published document by the Centers for Disease Control and Prevention (CDC) offers a precise definition of multidrug-resistant (MDR) isolates: their transformation from one or more antibiotics in three or more antibiotic categories to resistance. This definition is recognized internationally. XDR is defined as the ability of bacterial isolates to withstand at least one agent from all antimicrobial categories, with the exception of two or fewer. This means that they are susceptible to only one or two categories.

Biofilm Formation Quantitative Assay

Biofilm formation is quantified using a colorimetric microtiter technique (Spectrophotometric method) [14]. The assay involved the following steps:

1. A bacterial suspension was adjusted to a turbidity of 0.5 McFarland in Brain Heart Infusion (BHI) broth with 1% glucose, achieving a concentration of 1.108 CFU/mL.
2. The suspension was diluted to 5.106 CFU/mL (1/20 dilution) and further to 5.105 CFU/mL (1/10 dilution) by mixing 180 μL of BHI with glucose and 20 μL of the bacterial suspension in a sterile 96-well polystyrene microplate.
3. The microplates were incubated at 37°C for 24 hours to allow biofilm formation.
4. After incubation, wells were washed twice with phosphate-buffered saline (PBS) to remove non-adherent cells.
5. Adherent biofilms were stained with 100 μL of 0.1% crystal violet for 15 minutes.
6. Wells were washed twice with PBS to remove excess stains.
7. The crystal violet-stained biofilms were dissolved with 150 μL of 95% ethanol, and the absorbance was measured at 570 nm using a microplate reader.

The optical density cutoff value (ODc) was used to categorize isolates as biofilm producers or non-producers.

Molecular Analysis

DNA extraction

The DNA extractions from all samples of pathogenic Acinetobacter were conducted using the Bosphore extraction versatile Spin Kit (Anatolia genworks-Istanbul, Turkiye), following the manufacturer's instructions. The liquid portion containing DNA was used as a template for multiplex polymerase chain reaction (mPCR). The DNA quality was assessed by quantifying the A260/A280 ratio using an ultraviolet spectrophotometer and determining its integrity using agarose electrophoresis. The genomic DNA (gDNA) of the strains mentioned above was preserved at a temperature of -40 °C until it was defrosted on ice just before analysis.

DNA Amplification and Detection

The method of multiplex PCR was employed to detect the presence of the following resistance genes, namely carbapenem resistance OXA-23, OXA-48,OmpA and bla-TEM, as described in earlier studies. The primers, temperature and cycling settings utilized were as follows: for the bla-TEM gene (forward primer: 5'GAGACAATAACCCTGGTAAAT-3', reverse primer:

5'AGAAGTAAGTTGGCAGCAGTG-3') [15], OXA-23 (forward primer: 5'-GATCGGATTGGAGAACCAGA-3', reverse primer: 5'ATTTCTGACCGCATTTCCAT-3')(3), and OXA-48 (forward primer: 5'GCGTGGTTAAGGATGAACAC-3', reverse primer: 5'-CATCAAGTTCAACCCAACCG-3') [16]. (forward primer: 5' -ATTCGAATTCGCTACTATGCTTGTTGCTGCT -3', reverse primer: 5'-ATGTAAGCTTCGCTTCTTGACCAGGTTGAAC -3') for OmpA gene [17]. The amplification condition for bla-TEM and AbOmpA involved an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 95°C for 1 minute, 56°C for 45 seconds, and 72°C for 1 minute. The amplification was concluded with a final extension at 72°C for 7 minutes. For OXA-23 and OXA-48 the process begins with an initial denaturation at a temperature of 94°C for 5 minutes. This is followed by 35 cycles, each consisting of a denaturation step at 94°C for 30 seconds, an annealing step at 56°C for 45 seconds, and an extension step at 72°C for 1 minute . An initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 60.9°C for 45 seconds, and 72°C for 1 minute , final extension at 72°C for 7 minutes the condition of AbOmpA Gene. To detect amplified genes, 10 µL of each reaction was analyzed using gel electrophoresis on a 2% agarose gel that included ethidium bromide (5 µg/mL). The gel was exposed to an electric field of 100 V for 1 hour in a buffer solution with a concentration of 0.5 × TBE. The amplified DNA bands were then seen using a UV transilluminator. The size of the blaTEM amplicon was 459 base pairs (bp), size of OmpA was 1043bp ,the size of the OXA-23 amplicon was 501 bp, and the size of the OXA-48 amplicon was 438 bp.

Analysis of statistical data

The data analysis was conducted using the IBM SPSS-29 statistical tool (IBM Statistical Packages for Social Sciences, version 29, Chicago, IL, USA). The data was presented using fundamental statistical metrics such as frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The statistical significance of differences in different percentages (qualitative data) was assessed using the Pearson Chi-square test (χ^2-test), with the application of Yate's correction or Fisher Exact test as necessary. A significance level of 0.05 or lower was employed to establish statistical significance.

RESULT

Prevalence of *Acinetobacter baumannii*

From the total of 120 clinical samples collected from burn victims, 40 (33.3%) were identified as *Acinetobacter baumannii*. This high prevalence highlights the significant burden of *A. baumannii* infections in the burn units of Ramadi and Falluja Teaching Hospitals. The isolates were evenly distributed between the two hospitals, indicating a widespread issue rather than a localized outbreak.

Demographic Distribution of *Acinetobacter baumannii* Infections.

The demographic distribution of *Acinetobacter baumannii* infections among burn patients in the two hospital units is detailed in (Table 1). This table provides a breakdown of infections by age group and gender across the two hospitals: Al-Ramadi Teaching Hospital and Falluja Teaching Hospital. These findings highlight that *A. baumannii* infections affect both genders and various age groups similarly across the two hospitals, indicating a widespread issue that is not confined to a specific demographic within these units.

Among the 40 A. baumannii isolates analyzed, 18 (45%) exhibited moderate biofilm formation, with 10 isolates (45.45%) from Al-Ramadi Hospital and 8 isolates (44.4%) from Falluja (Table 2).

Hospital. Strong biofilm formation was observed in 22 (55%) of the isolates, with 12 isolates (54.54%) from Al-Ramadi Hospital and 10 isolates (55.5%) from Falluja Hospital.

The statistical analysis using Pearson's chi-square test revealed no significant difference in the biofilm formation capacity of isolates from the two hospitals (P = 0.949). This indicates that the ability of A. baumannii to form biofilms is consistent across isolates from both hospital units. These results highlight the pervasive capability of A. baumannii to form biofilms, irrespective of the hospital environment. The high proportion of strong biofilm-forming isolates underscores the challenge in

managing these infections, as biofilms contribute significantly to the resistance against antibiotics and the persistence of infections in hospital settings.

Antibiotic Resistance Patterns

The antibiotic resistance patterns of the 40 A. baumannii isolates were analyzed. Out of these, 34 (85%) exhibited multidrug resistance (MDR), defined as resistance to one or more antibiotics in three or more antibiotic categories. Additionally, 6 (15%) were classified as extensively drug-resistant (XDR), meaning they were resistant to all but one or two categories of antibiotics.

Resistance Rates:

- **Cefotaxime**: The highest resistance rate was observed for Cefotaxime, with 36 out of 40 isolates (90%) showing resistance.
- **Ciprofloxacin**: Resistance to Ciprofloxacin was also significant, with 30 isolates (75%) resistant.
- **Ampicillin/Sulbactam**: 32 isolates (80%) were resistant to ampicillin/sulbactam.
- **Piperacillin/Tazobactam**: 28 isolates (70%) showed resistance.
- **Cefepime**: Resistance was observed in 24 isolates (60%).
- **Amikacin**: 22 isolates (55%) were resistant.

Conversely, Colistin demonstrated the highest sensitivity among the isolates, with only 4 isolates (10%) showing resistance. This finding underscores the continued effectiveness of Colistin as a last-resort treatment for MDR *A. baumannii* infections (Figure 2).

Molecular Prevalence of OXA-23, OXA,48, OmpA and blaTEM Gene among carbapenem -Resistant Acinetobacter Isolates.

Out of the 40 A.baumannii isolates that produce carbapenem, 32 (80%) had the OXA-23 gene, 26(65%) had OmpA gene ,22 (55%) had blaTEM gene and none of the isolates had the OXA-48 gene (Figure 3and 4), respectively. The data demonstrates that the prevalence of the OXA-23 gene was higher compared to that of the OmpA,bla-TEM and OXA-48 genes.

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DISCUSSION

In this study, we focused on *A.baumannii* bacteria infection that may isolated from burn wounds in the Al-Anbar government (Al-Ramadi Teaching Hospital Center and Al-Faluja Teaching Hospital Center) because Outbreaks resulting from *A. baumannii* are frequent in intensive care (ICU) and burn units [18] and Patients who have sustained burn injuries are especially vulnerable to *A. baumannii* infection while hospitalized, according to several studies. It has been reported that this bacterium ranks second in the frequency of nosocomial infections among patients who have sustained burn injuries [21].

Out of 120 patients who have burn wounds in two centers,40(33.33%) have *A.baumannii* infections; this percentage agrees with many studies performed in Iraq, which found that *A.baumannii* represents the second most frequent bacterial type isolated from the burn [21].

A. baumannii has grown prevalent in hospitals due to its versatile genetic machinery, which allows it to quickly evolve resistant elements and its excellent ability to withstand demanding environments. The rising incidence of multidrug resistance in *A. baumannii* strains has led to *A. baumannii* becoming the most significant pathogen, second only to Pseudomonas aeruginosa, among non-fermentative gram-negative bacteria in a burn unit [22]. The two most concerning issues are the rise of *Acinetobacter baumannii* strains resistant to all commercially available antibiotics and the fact that new antimicrobial agents still need to be developed. As a result, there is a limited selection of antibiotics that can be used to treat *Acinetobacter baumannii* isolates that have developed resistance to multiple drugs [23]. In this study, *A.baumannii* shows 85% MDR. The proportion of MDR isolates agreed with two studies performed in Iraq [24,25], which observed that multidrug-resistant organisms were prevalent among burn injury patients in Iraq's northern regions. Antimicrobial resistance in clinical *A. baumannii* isolates is influenced by several factors. One significant reason is the presence of several resistance genes in MDR *A. baumannii* strains. Additionally, horizontal gene transfer between different polyclonal MDR *A. baumannii* strains is possible [26].

The prevalence of antibiotic resistance in *A. baumannii* is concerningly rising, resulting in heightened morbidity, mortality, and treatment expenditures within intensive care unit and burn unit environments, according to surveillance studies conducted in recent years [27]. Conversely, this

study showed that *A.baumannii* is highly sensitive to Colistin, which is in agreement with a study performed in Iran [28].

ESBLs originate from mutations that change the amino acid arrangement surrounding the active site of the narrow-spectrum beta-lactamases (TEM 1, TEM 2, or SHV 1) genes. Plasmids are easily transferable between bacterial species and are usually responsible for encoding them [29].

The identification of the blaTEM gene at the molecular level in this research may lead to resistance against cephalosporins, such as ceftazidime, cefazolin, cefoxitin, and Cefotaxime. As a result, it is accountable for the formation of phenotypic extended-spectrum β-lactamases [30]. Strains of A. baumannii produce β-lactamases encoded by the blaTEM gene, which are transported on plasmids and may contribute to the organism's overall longevity and long-term viability. The TEM type β-lactamases are derived from the enzymes TEM-1 and TEM-2. TEM-1 is a highly significant β-lactamase enzyme that is commonly found in Gram-negative bacteria. More than 130 TEM enzymes have been identified, utilizing a valuable mechanism to spread resistant genes [31]. In this study, 22 (55%) isolates had the blaTEM gene. The current findings disagree with Ghaima's results [32], which detected the positive bla_{TEM} 75%.while another study by Ibrahim et al [33], reported 78.9 % of isolates carried bla_{TEM}. On the other hand, our finding agreed with a study performed by Asgin *et al* [34], which reported 53.6% of isolates had this gene.

OmpA is a type of protein called porin, which is found in the outer membrane. The AbOmpA, a trimeric porin, plays a role in solute transport and pathogenicity in A.baumannii [34]. OmpA contributes to disease through many mechanisms, including apoptotic induction, immunomodulation, cell adhesion and invasion, biofilm formation, and antimicrobial resistance. AbOmpA can cause apoptosis in dendritic cells by specifically targeting the mitochondria. The clinical manifestation of A. baumannii infection is characterized by the overexpression of the ompA gene, which is directly linked to the development of pneumonia and bacteraemia, as well as increased patient mortality(36). In this study, 26(65%) of isolates were positive for the OmpA gene, and all 22 isolates which were strong biofilm formation showed positive OmpA this may interpret the strong relation between this gene and biofilm formation this agreement with the study performed in Iran [17] and South Korea [37] in study performed in Iraq on burn wounds 10 (80%) isolates of

A.baumannii were positive for OmpA this variation may attributed to the small samples size of their study [38].

Carbapenem resistance in A. baumannii encompasses the development of various mechanisms, including the production of carbapenemase enzymes, alterations in membrane permeability, changes in penicillin-binding proteins, and enhanced expression of efflux pumps. Acinetobacter spp. Commonly develop resistance to carbapenem antibiotics through the emergence of OXA-type carbapenemase and metallo-β-lactamases (MBLs) [39]. The class D carbapenemases are a significant cause of carbapenem resistance in Acinetobacter. Among these, OXA-23-like and OXA-24-like carbapenemases have frequently been identified as the most common types in numerous nations [40]. Out of the 40 A.baumannii isolates, 32 (80%) had the bla OXA-23 gene. This percentage agrees with the many studies first performed in Pakistan [41], second in Iran [3] that found a high prevalence of the gene was 85.1%.and in Tunisia [42].

The appearance of the OXA (oxacillinase) group of β-lactamases (Class D) has led to several challenges in managing and treating opportunistic infections. The blaoxa48 gene is highly prevalent in K. pneumoniae and serves multiple crucial functions, including the facilitation of biofilm formation and the development of resistance against carbapenems [43]. To date, the blaOXA-48 gene has not been identified in P. aeruginosa isolates; only a few studies have reported OXA-48 in A. baumannii [39].

In this study, non-isolates had the bla_{OXA-48} gene. These findings were in agreement with those of Cheikh et al [40], and Boral et al [44], who stated that the bla_{OXA-48} gene was not present in any of the A. baumannii isolates. The bla_{OXA-48} and blaGES-2 genes were not present in any of the A. baumannii isolates, as reported by Romanin et al [45]. On another hand, a study in Iran showed that 92% of isolates had the bla_{OXA-48} gene [46]. These findings indicated that the prevalence of bla_{OXA-48} may differ among countries.

Bacterial biofilm formation is a significant pathogenicity factor that inhibits the effectiveness of antibiotics and immune defense responses [47]. *A. baumannii* has acquired numerous virulence factors and is accountable for causing severe, life-threatening infections. The capacity to create biofilms and the existence of various adhesins contribute to the emergence of infections and the

resilience to antimicrobial drugs(48).in this study, *A.baumannii*, shows the formation of biofilm in all isolates this agreement with two studies performed in Iraq [49,50].

CONCLUSION

Acinetobacter baumannii remains a significant challenge in burn units due to its high rates of multidrug resistance and strong biofilm-forming ability. Effective management of A. baumannii infections requires a multifaceted approach, including stringent infection control measures, judicious use of antibiotics, and ongoing research into novel therapeutic strategies. The findings from this study contribute to the growing body of evidence needed to tackle this formidable pathogen and improve patient outcomes in burn units and beyond.

Limitations of study

The study included patients from the Burn Unit of the Al-Anbar Government, which must be taken from units in all burn centers of Iraq country. Furthermore, the duration of the investigation may be short, and we suggest making the study long enough to include more samples.

Ethical consideration

This study was approved by the Medical Ethics Committee of the University of Al-Anbar Governorate in Ramadi, Iraq, following the Helsinki Declaration. All research participants, including patients and their parents, provided signed informed consent (dated 3/12/2023).

Disclosure: The authors have disclosed that they have no significant relationships with,or financial interest in, any commercial companies pertaining to this article

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported inthis paper.

AUTHOR'S CONTRIBUTIONS

Noor Nadhom Khalaf: Conceptualization, Methodology, Software, Data curation, Writing-Original draft preparation. **Mohammed Jasim Mohammed**: Visualization, Investigation. ***Shaymaa Hafez Maiteb****:* Supervision, Software, Validation, Writing- Reviewing and Editing,

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Tables:

Table 1: The number and percentage of *A. baumannii* isolated from burn patients in two units

		Hospital Admission cite		Total	P Value
		Al-Ramadi Hospital	Al-Faluja Hospital		
Age year	21-30 n(%)	7 (31.8)	10 (55.5)	17	0.294
	31-40 n(%)	7 (31.8)	3 (16.6)	10	
	41-50 n(%)	8 (36.3)	5 (27.7)	13	
Gender	Male n(%)	10 (45.45)	7 (38.8)	17	0.676
	Female n(%)	12 (54.54)	11 (61.1)	23	

*Significant difference between percentages using Pearson Chi-square test (X2-test) at 0.05 level.

Table 2: The percentage of biofilm formation

Biofilm formation	Al-Ramadi Hospital	Al-Faluja Hospital	Total	P value
Moderate n(%)	10 (45.45)	8 (44.4)	18	0.949
Strong n(%)	12 (54.54)	10 (55.5)	22	

*Significant difference between percentages using Pearson Chi-square test (X^2-test) at 0.05 level.

Figures:

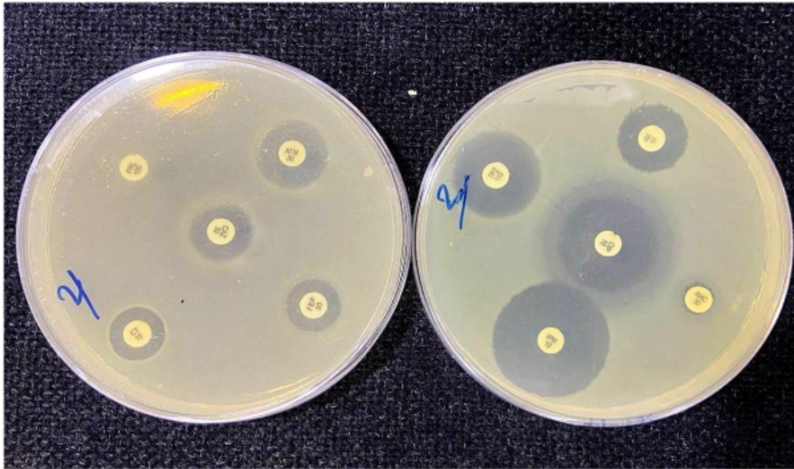

Figure 1: Antibiotic susceptibility test of A.baumannii shows the zone of inhibition on Mueller-Hinton Agar.

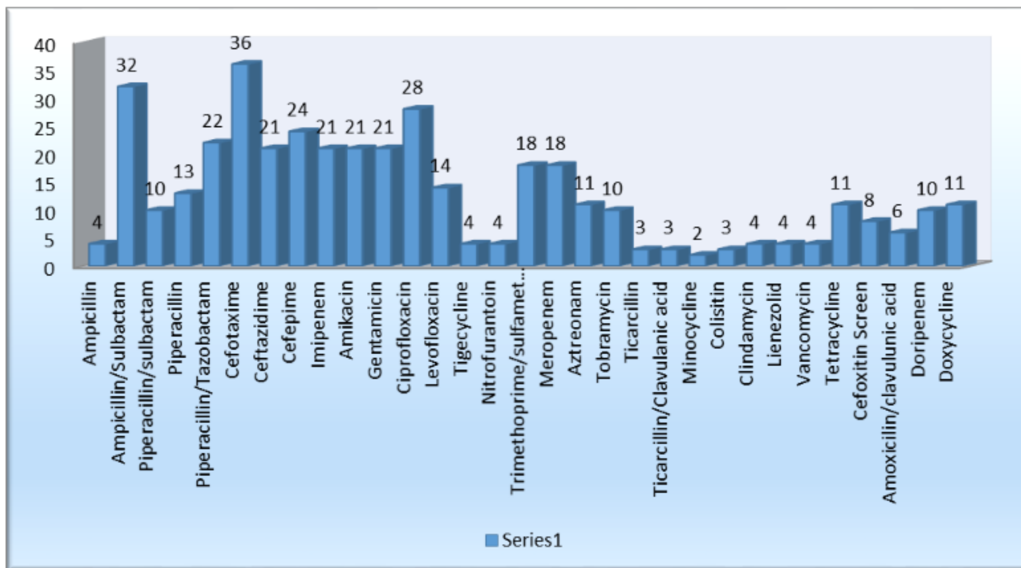

Figure 2: Percentage of *Acinetobacter baumannii* resistance to antibiotics and multi-resistance.

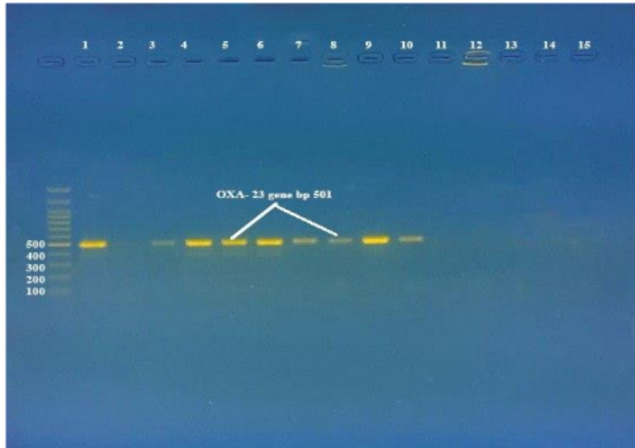

Figure 3: Agarose gel electrophoresis represents 501 bp OXA-23 gene in Acinetobacter Species.

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Figure 4: Show the Prevalence of OmpA 1043bp gene in A.baumannii isolates.