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Research Article

Characterisation of Antibiotic Genes of *Acinetobacter baumannii* Isolated from Patients in Baghdad Hospitals

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A B S T R A C T

Introduction: The gram-negative bacterium known as *Acinetobacter baumannii* is frequently found in soil and water, as well as in samples taken from animals and humans. This study isolated and identified 50 urine samples from four Baghdad educational hospitals. Method: Biochemical testing, selective synthetic media (CHROMagar), and polymerase chain reaction identified all bacterial samples. Only 20 isolates were identified as A. baumannii using a primer targeting the blaOxa-51 gene. Antibiotic susceptibility was tested using disc diffusion with eight antibiotics.

Results and Conclusion: The susceptibility testing of A. baumannii to antibiotics revealed that this bacterium had a higher resistance towards cefotaxime (8, 40%), followed by imipenem (6, 30%), gentamycin (5, 15%), and trimethoprim (2, 10%). The resistance was the same for both tetracycline and tobramycin (3, 15%). In contrast, the antibiotics ampicillin-sulbactam and doxycycline encountered a lower level of resistance, specifically 1% and 5% respectively. Analysis of 20 DNA samples of A. baumannii revealed the presence of *OmpA and CsuE* genes, with a gene size of 168 bp and 162 bp, respectively. The percentage of these genes in the samples was found to be 60%.

Keywords: Acinetobacter baumannii, Antibiotics, Resistance, Genes, AMEs

Introduction

The gram-negative bacterium known as *Acinetobacter baumannii* is frequently found in soil and water, as well as in samples taken from animals and humans.[1,2] The development of endocarditis, infections of the skin and soft tissues, meningitis, urinary tract infections (UTIs), and pneumonia are all significantly influenced by these microorganisms.[3,4] The fact that these bacteria are opportunistic microorganisms with innate characteristics that enhance their resistance to a wide variety of medicines is directly responsible for the quick emergence of strains that are resistant to many medications and even extremely resistant to certain pharmaceuticals.[5] Acinetobacter spp. are gram-negative bacteria that are obligate aerobes, non-motile, coccobacilli, non-fermenting, and non-fastidious. They are often observed in diploid formations or chains of different lengths. Additionally, they have

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catalase-positive characteristics and do not show oxidase activity.[6] Acinetobacter spp. is a type of bacteria that is commonly found in hospitals and can cause infections such as pneumonia, bloodstream infections associated with catheters, and various clinical illnesses, especially wound infections. It is the most common species responsible for infections and has a patient mortality rate of up to 60%.[7] Biofilms are microbial aggregations that are created by the microbes themselves and can be found on biotic or abiotic surfaces. They contain exopolysaccharide matrices.[8] Furthermore, *A. baumannii* is capable of producing biofilms on a wide range of surfaces, including biotic surfaces such as host epithelial cells as well as abiotic surfaces such as stainless steel and polypropylene.[9] Through the establishment of biofilms, numerous drug resistance mechanisms, such as drug exclusion, permeability defeat, and enzymatic degradation processes, are improved.[10] Evidence indicates that the *A. baumannii* genome harbours a diverse array of virulence factors that are linked to bacterial cell adhesion and have a profound impact on the production of biofilms.[11] Colquhoun and Rather have reported that *OmpA* and *CsuE* proteins in *A. baumannii* exhibit the highest degree of conservation, with a presence rate ranging from 81% to 100%.[12] Acinetobacter is quite common in hospitals because it can live on dry and moist surfaces.[1] It can cause pneumonia, endocarditis, meningitis, skin and wound infections, peritonitis in peritoneal dialysis patients, urinary tract infections, and bloodstream infections. These diseases are typically associated with endotracheal tubes or tracheostomies.[2,3] Acinetobacter's antibiotic susceptibility pattern might vary greatly regionally and over time between several hospital departments. In order to select the most suitable therapy, it is essential to regularly check these pathogens because of the variations in the Acinetobacter resistogram.[4,5] It is crucial to identify the common susceptibility profiles due to the diverse multidrug resistance patterns demonstrated by clinical isolates of Acinetobacter. Isolating Acinetobacter from urine samples was the primary goal of this investigation, which aimed to identify them using a simpler method. The susceptibility of these isolates to different antibiotics was then evaluated.

Material and Method

Bacterial Isolates

During a three-month period from February to April 2023, a total of 50 urine samples were collected from patients at four distinct hospitals in Baghdad, namely Al-Karama, Al-Yarmouk, Al-Kathmyaa, and Al-Mohammadia. Following cultivation on MacConkey's agar (HiMedia, India) and overnight incubation at 37 °C, isolates were obtained from clinical samples. The non-lactose fermenting bacteria, which were either colourless or slightly brownish, were subsequently transferred to new cultures and cultivated for consecutive overnight periods. The identification was conducted based on morphological and biochemical analyses.[13,14] The putative bacterial isolates, characterised by gram-negative coccobacillary or diplobacillary cells and negative oxidase results, underwent additional identification using conventional biochemical testing.

Extraction of Genomic DNA

Genomic DNA was isolated from the bacterial growth using the ABIOpure Extraction process to serve as a template for PCR amplification. The amplification reaction mixture (25 µl) contained 12.5 µl of GoTaq® Green Master Mix 2X (Promega, USA) and 2.5 µl (10 M) of forward (GCCATAACCAACACGCTTCA) and reverse (GGCAACCCCACAGAAGTAT) primers for each gene (Kapa, USA). A DNA thermal cycler, GeneAmp 9700 Thermal Cycler made by the Singaporean firm Applied Biosystem, was used to amplify the 16S ribosomal RNA. Denaturation was first performed at 95 °C for 3 minutes. Then, there were 30 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute, and finally, an extension step at 72 °C for 5 minutes. The PCR amplicons that were created were analysed using electrophoresis and a gel documentation system called BioDocAnalyze Live, manufactured by Biometra Biomedizinische Analytik GmbH in Germany. The ABIOpure extraction procedure was employed to extract genomic DNA from the bacterial growth for subsequent use as a template in PCR amplification.

Antibiotic Susceptibility Test

The disc diffusion method on Muller Hinton agar (Mast, UK) was employed to determine the resistance of bacterial isolates to various antibiotics, following the guidelines set by CLSI in 2020.[15] The antibiotics used in the study were ampicillin-sulbactam (10 µg), cefotaxime (30 µg), imipenem (10 µg), gentamycin (10 µg), tobramycin (10 µg), tetracycline (30 µg), doxycycline (30 µg), and trimethoprim (2 µg). The bacterial isolates were introduced into the culture medium using the streaking technique and were thereafter placed in an incubator at a temperature of 37 °C for a duration of 24 hours. The samples were then examined and analysed.

Detection of Genes Responsible for Biofilm Formation

The detection of *OmpA and CsuE* genes was conducted using a designated set of primers as indicated in Table 1. The detection was performed by amplifying the *OmpA* and *CsuE* genes in the DNA template isolated from *A. baumannii* using a reaction mixture with a volume of 25 µl for each gene. The PCR amplification products were subjected to electrophoresis on a 2.5% agarose gel.

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Table 1.Sequence of Specific Primers

Names of Genes	Sequence of Genes 3 – 5	Product Size (bp)
OmpA	TGCTCCACAACCACAAGAGT GGCAACCACCACAGAAGTAT	162
CsuE	CATCTTCTATTTCGGTCCC CGGTCTGAGCATTGGTAA	168

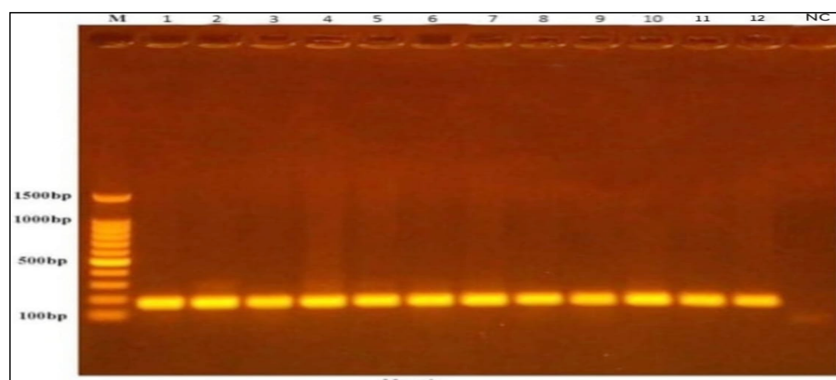
Results and Discussion

According to the data, only 20 out of the 50 samples were identified as *A. baumannii* isolates. Table 2 displays the distribution of the observed *Acinetobacter baumannii* in the four hospitals located in Baghdad's different regions.

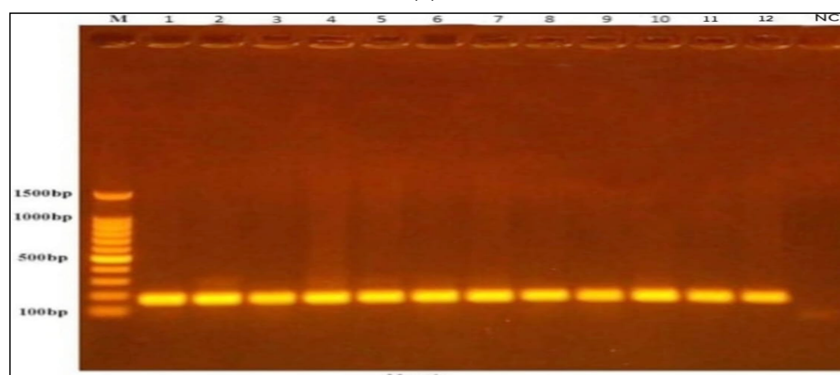
Table 2.Percentage of A. baumannii Bacteria in Different Educational Hospitals

Regions	n (%)
Al-Karama	5 (25)
Al-Yarmouk	10 (50)
Al-Kathmyaa	3 (15)
Al-Mohammadia	2 (10)

Based on the CHROMagar results, 20 out of the 50 bacterial isolates were determined to be A. baumannii. The microscopy and biochemical examinations identified the presence of gram-negative cocci exhibiting catalase-positive properties and yielding negative results in oxidase and nitrate reduction assays. In addition, the isolates showed negative results for the Simmons citrate test and positive results for the indole test. The results of biochemical and morphological research are consistent with the findings of Falah and his colleagues who successfully identified *A. baumannii* from burn wound swabs taken from patients at a prominent burns hospital in Tehran.[16] Moreover, our results are also in agreement with those of a study in which the existence of the pathogen (44.48%) was accurately detected in samples of sputum, pus, blood, and urine.[17] In order to confirm the identity of these 20 isolates as A. baumannii, a molecular identification method was carried out. The process entailed amplifying a particular gene consisting of 187 base pairs. The results unveiled a clearly distinguishable band, as illustrated in Figure 1.



(a)



(b)

Figure 1(a and b).Findings from 20 Bacterial Samples Aliquoted on 1.5% Agarose Gel Electrophoresis for BlaOXA Gene Amplification Traced using a 100 bp ladder marker and Stained with Eth. Br. M. Lanes 1-B Resemble PCR Products

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Table 1 shows the primers used for the PCR amplification of the blaOxa-51 gene. They were applied to 60 DNA samples obtained from different bacterial isolates. All isolates of A. baumannii were found to be positive for the blaOxa-51 gene and showed a clearly visible band with a molecular size of 178 bp on a 2.5% agarose gel, using an electric field strength of 5 volts/cm^2 for a duration of 90 minutes. Figure 1 depicts the results of gel electrophoresis. The blaOXA-51-like gene was detected in all isolates examined in the present study. This gene displays a broad distribution and is an inherent trait of the A. baumannii species.[12] Previous studies have shown that the blaOxa-51 gene has not been identified in any other Acinetobacter species, and is only present in every clinical isolate of A. baumannii.[15] The results of our research are consistent with a study that employed PCR to validate the existence of the blaOXA-51 gene, which acts as an indicator for recognising A. baumannii. The investigation found that out of the total isolates, 80 accounting for 97.56%, had the target gene.[16] The A. baumannii isolates demonstrated resistance to ampicillin-sulbactam, cefotaxime, imipenem, gentamycin, tobramycin, tetracycline, doxycycline, and trimethoprim.

The results of susceptibility of A. baumannii showed that these bacteria have a higher resistance to cefotaxime (8, 40%), followed by their resistance to imipenem (6, 30%), gentamycin (5, 15%), and trimethoprim (2, 10%). The resistance percentage was the same for tetracycline and tobramycin (3, 15%). On the other hand, lower resistance was observed to antibiotics ampicillin-sulbactam and doxycycline (1, 5%). The highest resistance was recorded to cefotaxime. This observation aligns with previous reports that observed a heightened resistance of the isolates to this antibiotic.[12,18] In addition, another study documented a remarkably high level of resistance to cefotaxime, reaching 92%.[19] The current investigation demonstrated a 30% resistance to imipenem. This finding is consistent with the research conducted by Yang and his colleagues, which revealed a 28% prevalence of antibiotic resistance.[20] However, it contradicts the findings of the study conducted by Anane et al., in which the resistance to antibiotics reached 83%.[21] The prevalence of gentamycin resistance in the current investigation was 15%. However, the resistance to gentamycin was shown to be 86% in another investigation.[22] The prevalence of A. baumannii resistance to tetracycline and tobramycin was below 15%, consistent with the findings reported by El-Badawy et al.,[23] who observed that A. baumannii demonstrates resistance to many antibiotics, including tobramycin (59%). Conversely, the occurrence of tetracycline resistance varied in different studies, with one reporting a rate of 62% and another showing an increase to 93%.[24] The prevalence of resistance to this antibiotic has shown a notable decrease, declining from 90.2% in 2010 to 69.14% in 2015, as reported by Dafopoulou et al.[25] On the contrary, Abdallah et al.[26] unequivocally demonstrated that the resistance rate for this drug was 100%. However, Maraki et al. conducted more investigations and discovered a resistance rate of 63.57%, which can be considered relatively high.[27]

Due to lack of availability, clinicians had to depend on older antibiotics such as trimethoprim-sulphamethoxazole. Furthermore, the research conducted by certain scientists demonstrated that ampicillin-sulbactam resistance was quite high (77%), while another study showed a resistance of 46%.[28] Articles by Lukovic et al. and Safari et al., however, showed that the percentages of ampicillin-sulbactam resistance were 31% and 59.1%, respectively.[29,30] It was found that the percentage of tetracycline resistance was 62%, which was similar to the percentage found by another researcher (60%).[24] Contrary to a study conducted by Abd El-Baky et al. that showed a resistance of 93%, this study did not indicate a very high level of resistance to tetracycline.[31] With a sensitivity rate of 28%, all isolates exhibited moderate resistance to ceftriaxone (48%). Contrary to what Abbas-Al-Khafaji and Aubais-aljelehawy reported, our results showed that a small percentage of bacterial isolates were ceftriaxone-resistant.[32]

A study by Monfared et al. showed that the resistance to doxycycline was 96%.[33] The percentage mentioned here varies from the resistance rate observed in the present investigation, which documented a 46% resistance to the doxycycline drug. Nevertheless, the prevalence of doxycycline resistance reached 57% in another study.[34] Conversely, As and Priyadarshini conducted a study which revealed a low resistance (20%) to doxycycline.[35] During this study, the highest resistance was recorded to cefotaxime (84%). This result is consistent with that of Musyoki et al.[18] where they recorded a resistance rate of 87%.

The presence of β-lactamases, an enzyme that renders the beta-lactam ring inactive, is another element that contributes to the development of resistance to some antibiotics in A. baumannii. Aminoglycosides-modifying enzyme is an example of such an enzyme.[36] Three mechanisms of aminoglycoside resistance in A. baumannii have been identified: (1) the presence of aminoglycoside-modifying enzymes (AMEs) that lower the antibiotics' affinity for their targets; (2) the randomisation of target sites by 16S rRNA methyl transferases; and (3) the restriction of aminoglycoside uptake due to increased efflux pump activity or loss of permeability.[37] The two main processes that have been associated with tetracycline resistance are enzymatic inactivation and ATP-dependent efflux

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pumps. One of the biggest public health problems globally, particularly in poor nations, is the frequency of A. baumannii infection in various areas of hospitals. Nosocomial infections are caused by A. baumannii, a highly significant bacteria that is difficult to control because of its persistence in the hospital environment and resistance to many medications. The number of fatal infections in hospitals has been rising, and some scientists attribute this to infections caused by A. baumannii, particularly strains that are resistant to multiple drugs. Isolation of A. baumannii from a patient can suggest bacterial colonisation rather than infection, which further complicates efforts to determine the actual frequency of this microbe. Colonisation of patients by Acinetobacter species that are resistant to multiple antibiotics has been documented by numerous researchers.

Detection of the Prevalence of OmpA and CsuE Genes by PCR

Using a typical PCR approach and particular primers as specified in Table 1, 20 DNA samples taken from A. baumannii were molecularly detected for *OmpA and CsuE* genes. The samples were then subjected to gel electrophoresis using agarose gel (2.5% concentration), at 5 volts/cm^2 for 90 minutes. Figures 2 and 3 indicate the 168- and 162-base pair sizes of the CsuE and OmpA genes, respectively, in A. baumannii, and the results demonstrated that these genes comprised 60% of the total. According to the findings, biofilm generation ability was higher in A. baumannii isolates that carried the CsuE and OmpA genes. Both genes are required for biofilm development.

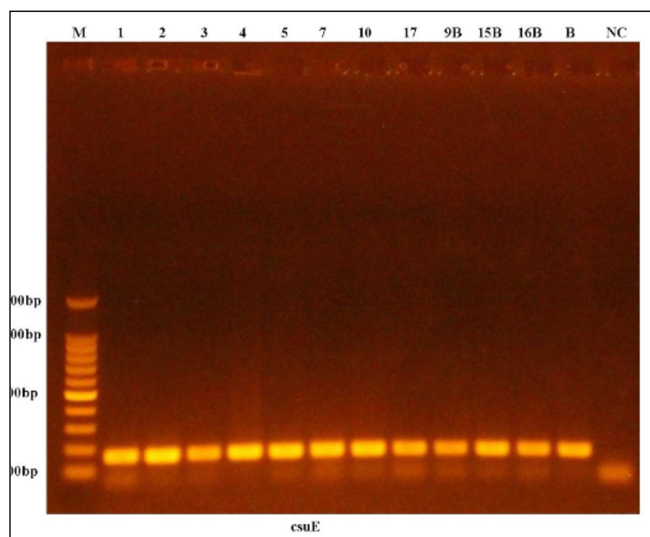

Figure 2. Results of the Amplification of CsuE Gene of Bacterial Samples Fractionated on 1.5% Agarose Gel Electrophoresis Stained with Eth. Br. M: 100 bp ladder marker. Lanes 1-B Resemble PCR products

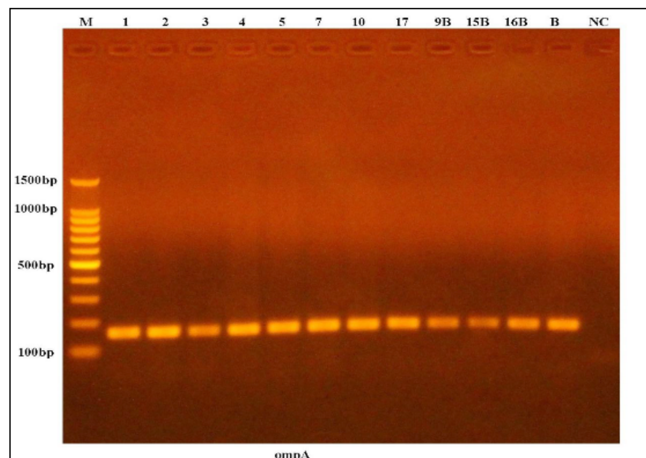

Figure 3.Results of the Amplification of OmpA Gene of Bacterial Samples Fractionated on 1.5% Agarose Gel Electrophoresis Stained with Eth. Br. M: 100 bp ladder marker. Lanes 1-B resemble PCR products

All biofilm producers, including some weak and moderate producers, had the CsuE and OmpA genes, according to our findings. In addition to these genes, 88% of isolates lacked the ability to form biofilms. This is likely due to a combination of factors, including genes related to biofilms and other physiological processes, as well as nutritional and environmental factors.[38] These results agreed with the work of Monfared et al. and Khoshnood et al.[33,39] which showed a high prevalence of biofilm-related genes OmpA and CsuE. Also, the CsuE gene was present in all isolates in a study,[40] but other studies showed the percentages of CsuE and OmpA to be 68% and 81%, respectively.[11] Despite the fact that there are numerous alternatives to antibiotics that function as antimicrobials against pathogenic germs, the changes in the above results can be due to the increased application of other drugs against pathogens, which is driven by the increased resistance of bacteria to antibiotics. These variations might have arisen as a result of seasonal, geographical, and unique solvent variations experienced.[40–45] An approach that involves detecting harmful microorganisms using Polymerase Chain Reaction (PCR) is used in several medical disciplines to determine the clinical aetiology associated with infectious diseases [42–45] and other harmful genetic disorders; it is regarded as an essential method in medicine.[46–57]

Conclusion

A. baumannii is responsible for various nosocomial infections. In this study, 20 out of 50 samples were identified as *A. baumannii* isolates. This study showed the highest resistance of this bacterium towards cefotaxime.

All biofilm producers, including some weak and moderate producers, were found to have the *CsuE and OmpA* genes.

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Conflict of Interest: None

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