ORIGINAL ARTICLE

Role of Pal and OmpA Genes in *Actinobacteria Baumannii* in Multiple Infections by Molecular Techniques

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ABSTRACT

Actinobacteria baumannii (*A. baumannii*) is a significant nosocomial pathogen known for its multidrug resistance and ability to survive in harsh environmental conditions. This bacterium has become a major concern in healthcare settings due to its involvement in various infections. Objectives: The aim of this study was to identify the parameters that were particularly linked to *A. baumannii* infections and to characterize the clinical symptoms. A sample of 180 patients from different sources (bloodstream, urinary tract, burn, and wound infections) whose *A. baumannii* was chemically and molecularly detected in a teaching hospital in Iraq. Molecular detection identified *Acinetobacter baumannii* in samples from the bloodstream, urinary, and wound burn infections. *A. baumannii* was detected in 150 of the 180 samples that were tested. 75% of these isolates had the pal gene and 80% had the ompA gene. *A. baumannii* was present in all infection types. The pal and ompA genes play significant roles in biofilm formation and immune evasion, highlighting their importance in the bacterium's virulence. *A. baumannii* was found in all infection types. The pal and ompA genes were commonly detected, underscoring their key role in the bacterium's virulence and persistence across different infection sites.

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Keywords: *Acinetobacter baumannii*; Biofilm Formation; ompA; Pal; Virulence Factors

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INTRODUCTION

A. baumannii is an opportunistic and nosocomial bacterial pathogen. It is a Gram-negative cocco-bacillus that is usually short, spherical, and rod-shaped (1,2). It is well-known for its resistance to multiple drugs and capacity to endure in severe environments (3). *A. baumannii* has become a ma-jor concern in healthcare settings due to its involvement in various infections (4,5).

Peptidoglycan-associated lipoprotein (Pal) and outer membrane protein A (OmpA) genes play a key role in its infections. All gram-negative bacteria have Omp that serve multiple functions in pathogenesis, catalysis, and passive and active transportation (6). OmpA causes mitochondrial dysregulations and dysfunctions after it attaches itself to the mitochondria (7). Pal and OmpA ena-ble this bacterium to infiltrate and disrupt the cells and their functions using the fastening mecha-nism (8). Pal and OmpA play a significant role as a virulence factor in human disease (6,9).

A. baumannii remains antibiotic-resistant due to its virulence potential and resistance to comple-ment. Antibiotic resistance to *A. baumannii* is worsening among Iraqi patients seeking medical treatment in Kuwait, Jordan, Iran, India, Lebanon, and Turkey (4). These bacterial infections and multidrug- resistance have been isolated in Malaysia recently (5,10). An efficient and multi-faceted approach to combating *A. baumannii* antibiotic-resistant is still limited. There is limited knowledge regarding their dynamisms and functions of Pal and OmpA in disease environments. This paper is the preliminary findings of the larger study.

The aim of this study was to identify the parameters that were particularly linked to *A. baumannii* infections and to characterize the clinical symptoms.

MATERIALS AND METHODS

Sampling

The target population of this study was 1800 patients in a teaching hospital in Iraq. A sample of 180 patients that were infected with *A. baumannii* from different sources (bloodstream, urinary, burn, and wound infections) was chemically and molecularly detected. The sample size represents 10% of the total population. The sample size was calculated using G*power software 3.1.9.7. A total of 45 samples from bloodstream, urinary, burn, and wound infections each were collected. This study obtained approval from the teaching hospital ethical committee before the sampling.

Identification of *A. baumannii*

The study identified isolated colonies from selective media using morphological characteristics (11), gram stain, and light microscope examination (12). The texture, color, and shape of the colo-nies that were observed following the bacterial growth on MacConkey agar and blood agar (13) were used to perform initial analysis of the isolates.

Biochemical tests

The study conducted biochemical tests on 88 isolates of chromagar bacteria to identify isolated colonies. The tests included an oxidase test (14), a catalase test (15), a urea production test (16), and a citrate utilization test (17). The tests were conducted to determine the presence of bubbles, the ability of the bacteria to break down urea, and the presence of citrate in the medium. Positive results indicated the presence of the catalase enzyme, while negative results indicated the absence of catalase activity.

Antibiotic susceptibility test

The Kirby-Bauer method was used to conduct the antibiotic susceptibility test, following CLSI (2021) guidelines (18). Cultured *A. baumannii* isolates were incubated at 37°C for 24 hours, then centrifuged and diluted to the McFarland turbidity standard. Antibiotic disks were placed on agar surfaces, and the inhibition zone's diameter was calculated. The inhibition zone, defined as the area around the disk with no visible growth, was compared to the standard value for each drug.

Molecular Analysis

The DNA was extracted using a Promega company kit, which involved adding nuclei lysis solu-tion, RNase solution, protein precipitation solution, and centrifugation. The DNA pellet was then rehydrated in rehydration solution for 1 hour at 65°C or overnight at 4°C. The purity and concen-tration of DNA solution were determined using a Nano drop spectrophotometer. The study exam-ined resistant *A. baumannii* isolates for the presence of arr2 and rpoB genes, sequencing the rpoB gene to identify mutations that make the bacteria resistant to rifampin. Primer preparation involved spinning the pellet, dissolving primer in nuclease-free water, and dilution to 10 pmole/µl for PCR. The total volume of the PCR mixture was 25 µl.

Agarose gel electrophoresis

A 1% agarose gel was prepared by mixing 50 ml of 1X TBE buffer and 0.5 g of agarose. The so-lution was cooled, then added to a comb and taped to a tray. The gel was then electrophoresed with 5 µl of each PCR product and five microliters of DNA ladder. The gel was then exposed to UV light for 1 hour to visualize DNA bands before photographed.

RESULTS

Descriptive Statistics

The majority participants of this study were patients with age ranges between 31-41 and 61-71 years old, while the patients with age ranges between 91-100 years old constituted the minority participants (Figure 1A). There were no participants with ages 81-91 years old. This showed that the *A. baumannii*-caused infections affected patients of all ages (Figure 1A). Therefore, age might be a key factor in the spread of the disease caused by this bacterium in the healthcare environment.

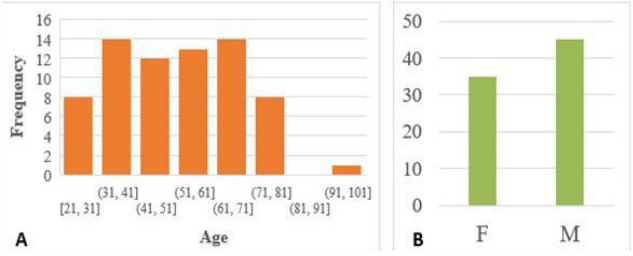

Figure 1 : (a) Distribution of patients by age and (b) Distribution of patients by gender

Gender was another factor observed in the patients with different infections caused by *A. baumannii*. The result of this study showed that male patients were more affected by *A. baumannii* than female patients (Figure 1B). This information might help us to comprehend the *A. baumannii* virulent factors. A recent study found that male patients in the 41-60 age range had significant *A. baumannii* infections (P≤0.005). In addition, there was no statistically significant difference in the overall incidence rate between both genders.

Identification of *A. baumannii*

Figure 2 displays the result of identification of A. baumannii in patient samples based on their (a) age and (b) gender. The results showed the presence of A. baumannii DNA isolates in all age groups and genders. It was present in mostly patients in their 30s and 50s, and in more males than females. It was noted that the patients with bloodstream infections and autoimmune diseases were more prone to *A. baumannii* compared to other infections.

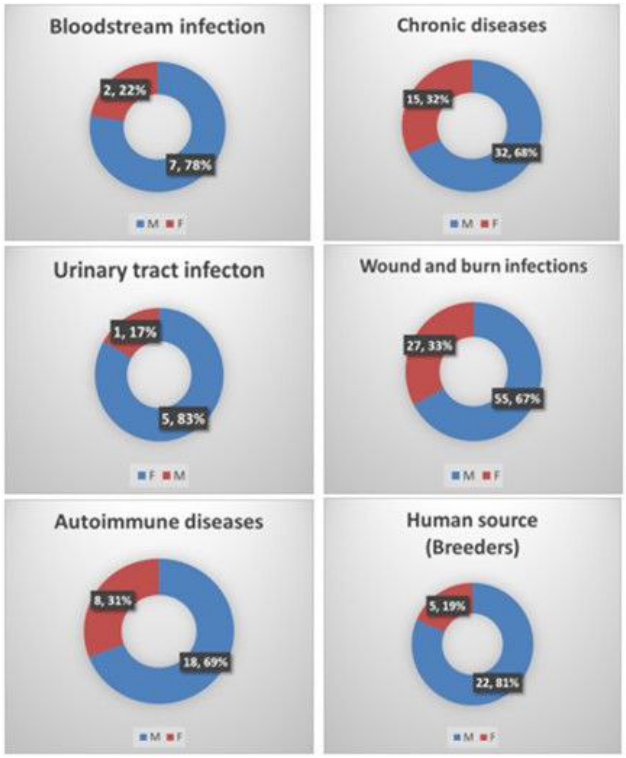

Figure 2: Result of identification of *A. baumannii* in patient samples based on their (a) Age and (b) Gender.

Biochemical Test

The results of the biochemical test for *A. baumannii* isolates is displays in Table I. The results showed that hemolysin production, lactose fermentation, urease production, indole production, and oxidase production were tested negative for all samples. However, catalase production and citrate utilization were tested positive for all samples. We observed that a catalase positive result was evi-dently indicated by the bubbles that form when oxygen gas is produced. This result indicated the presence of catalase in the *A. baumannii* bacterium.

Susceptibility Test

Figure 3 displays the results of the antibiotic susceptibility test. The results showed that A. bau-mannii successfully grew in Chromagar compared to MacConkey and Blood Agars. The chro-magar medium encourages the growth of *A. baumannii* while preventing the growth of other mi-croorganisms. The identification of this bacteria was

Table I: Results of biochemical test for *A. baumannii* isolates

Id	Parameters	Result
1	Hemolysin production	-
2	Catalase production	+
3	Lactose fermentation	-
4	Urease production	-
5	Citrate utilization	+
6	Indole production	-
7	Oxidase production	-

Note: Five of these parameters tested negatives, while the other two tested positives.

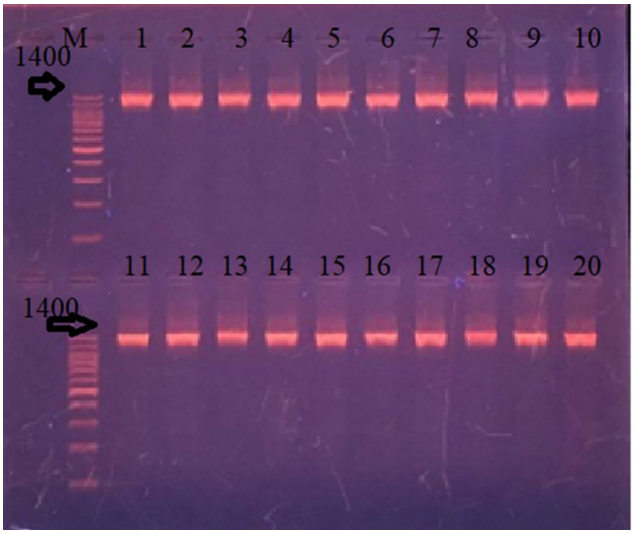

Figure 3: Results of gel electrophoresis.

achieved based on changes in the medium or the color of the colonies. The growth observed on these plates suggested that the bacteria were re-sistant to carbapenem after adding cefotaxime to the chromagar plates.

Figure 4 displays the results of the susceptibility test for *A. baumannii* isolates on ten different anti-biotics. The study found that all strains of *A. baumannii* were 100% resistant to all tested antibiotics (Figure 4). They showed nonsusceptibility to at least one agent in all antibiotic groups but two or fewer antimicrobials (100% were XDR). The resistance patterns were as follows: 100% resistance to cefotaxime, ceftriaxone, cefepime, rifampin, amikacin, ampicillin-sulbactam, trimethoprim-sulfamethoxazole, levofloxacin, doxycycline, and

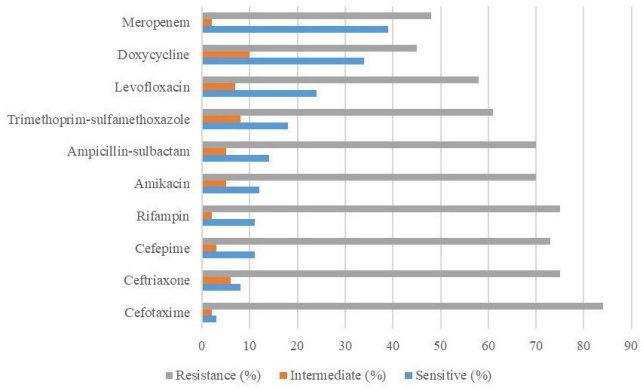

Figure 4: The susceptibility test of *A. baumannii* isolates on 10 different antibiotics. Note: Most of the tests showed higher resistance against *A. baumannii* isolates

meropenem. The cefotaxime, ceftriaxone, and rifampin were the most resistant, with a mean minimum inhibitory concentration (MIC) value of ≥17 µg/mL, followed by cefepime, ampicillin-sulbactam, and amikacin.

Gel Electrophoresis Analysis

In the further validation analysis, the results showed that the smaller bands were at the bottom of the gel (Figure 5), indicating bacteria DNA fragments. The large bands were at the top of the gel, which represented the *A. baumannii* DNA, and the finding indicated the presence of DNA in all the samples. This finding supported the results obtained in the biochemical test and the antibiotic susceptibility test.

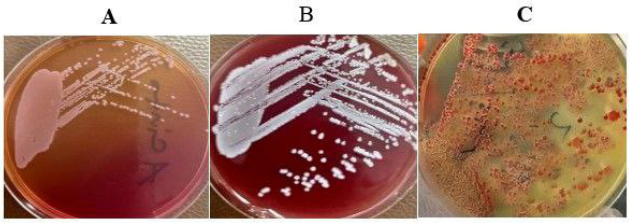

Figure 5: Results of antibiotic susceptibility test. Growth of A. baumannii on (A.) MacConkey agar, (B.) Blood Agar, and (C.) ChromagarTM incubated at 37°C for 24 hr.

Molecular Analysis

Figure 6 displays the results of molecular analysis. Molecular analysis revealed the presence of *A. baumannii* in bloodstream, urinary tract, and wound burn infection samples. Thirty samples tested negative, and 150 of the 180 samples examined produced positive results. Pal and OmpA genes with high homology to *A. baumannii* were identified. 75% of these isolates had the pal gene, and 80% had the ompA gene. The findings demonstrated that 70% of these isolates with bloodstream, chronic, urinary, wound, and burn infections and in human sources had the Pal gene. On the other hand, 80% of isolates with bloodstream, urinary tract, wound, and burn infections had the OmpA gene. The findings showed that the pal gene was found to be present in 69% and 81% of patients with autoimmune diseases and human sources, respectively.

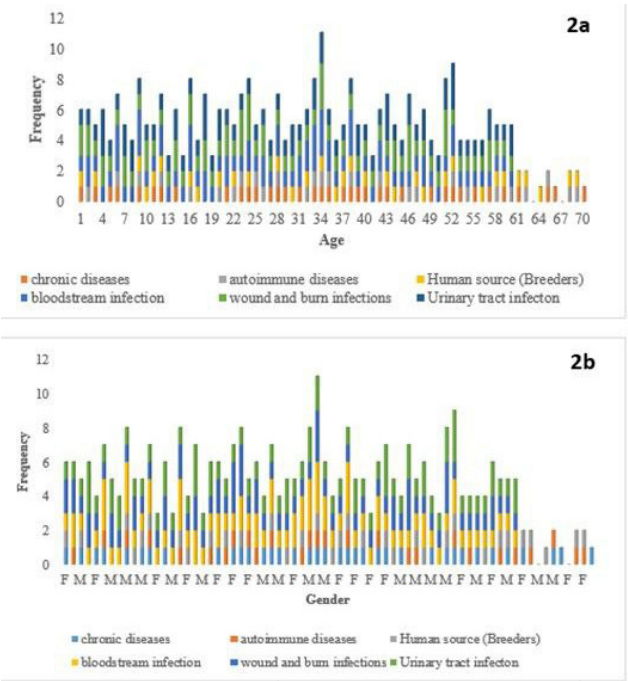

Figure 6: Comparison of A. baumannii infection frequency by age (2a) and gender (2b)

DISCUSSION

A. baumannii infections are observed in different age groups, with high frequencies in the 31-41 and 61-71 age groups. The absence of participants aged 81-91 might be because sample size limi-tations. Therefore, age might be a key factor in the spread of the disease caused by this bacterium in the healthcare environment. This can help us understand the characteristics of the virulent factors. Recently, (19) found that the older age is a risk factor for the mortality caused by *A. baumannii* bloodstream infections. This suggested that age-related infection patterns can guide targeted interventions for susceptible groups. This study also found that male patients were more prone to *A. baumannii* infections than their female counterparts, which is consistent with previous research (20). However, no significant difference was found in overall infections between genders. This might be due to gender-related biological or environmental factors that impact immune responses. *A. baumannii* is an opportunistic pathogen known for resistance to multiple antibiotics and preva-lence in nosocomial environments. Studies have previously reported that *A. baumannii* is highly resistant to many antibiotics (3-5,21).

The finding showed that the patients with bloodstream infections and autoimmune diseases were more prone to *A. baumannii* compared to other infections. Studies showed that the *A. baumannii* pathogen can cause infections in various sources, including the bloodstream, urinary tract, burns, wounds, and human interaction sources (22,23). Genetic variability in the bacteria can potentially expand its survival in hospitals. (24) have identified *A. baumannii* clones in hospital samples and reported nosocomial infections. Further analysis to validate these infections in various sources, the finding showed that catalase production and citrate utilization were tested positive for all infections. Generally applied to gram-negative bacteria, the catalase test can be used to differentiate catalase-positive strains of opportunistic and nosocomial bacteria from catalase-negative strains (25). The ability of *A. baumannii* to import citrate and use it as its only source of carbon and energy was in-dicated by the visible presence of growth on the medium and the change in pH indicator color caused by the elevated pH. According to (26), this type of organism is known as citrate positive.

The study found that 70% of isolates with bloodstream, chronic, urinary, wound, and burn infections had the

Pal gene, while 80% had the OmpA gene, and 69% and 81% had autoimmune dis-eases. Virulence factors, such as Pal and OmpA, can affect bacteria survival. *A. baumannii* strains of human origin can exhibit virulence-related factors, such as affecting cytotoxicity, persistence, etc. (8). The most prevalent protein in OMVs is OmpA, a conserved protein from *A. baumannii.* Pal is a lipoprotein associated with peptidoglycans that is essential to the integrity of the outer membrane (27). *A. baumannii* infection can be significantly prevented by a DNA vaccine that en-codes OmpA and Pal. OmpA and Pal, key *A. baumannii*'s virulence factors, may be a viable vac-cine target. According to immunoproteomics and reverse vaccine studies, Pal is a strong candidate for vaccine (28). Thus, OmpA and Pal are potent as vaccine candidates.

CONCLUSION

A. baumannii was found in all infection types. The pal and ompA genes were commonly detected, underscoring their key role in the bacterium's virulence and persistence across different infection sites. The pal and ompA genes play significant roles in biofilm formation and immune evasion, highlighting their importance in the *A. baumannii*'s virulence. OmpA and Pal functions and roles in *A. baumannii* pathogenesis and vaccine development are still unclear; future work can explore this area.

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