

Original Research Paper

Genetic study of *Omp A* , *Sur A1* and *bas D* genes produced by *Acinetobacter baumannii* which isolated from patients with kidney failure

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Article history

Received: 23/5/2025

Revised: 7 /6/2025

Accepted: 11/6/2025

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Abstract: Kidney failure patients suffer from the risk of developing gram negative infections, as the development of bacterial resistance to antibiotics is a major problem that limits the use of antibiotics to treat diseases resulting from bacteria. From this perspective, our research aims to isolate and diagnose *Acinetobacter baumannii* and ability to produce genes antibiotics resistance. **Methodology:** 102 specimens were collected from kidney failure patients in AL- Najaf Governorate. Iraq, from (September to November 2024). The types of gram-negative bacteria identified using biochemical testing and vitek 2 system . Specimens. All specimens were grown on MacConkey agar , using Selective media for *Acinetobacter*, CHROMagar *Acinetobacter* and Leeds *Acinetobacter* Medium. extracted using the *Wizard® Genomic DNA Purification Kit*, PCR testing was performed to detect genes. **Results:** Out of a total of 102 patients diagnosed with kidney failure, Gram-negative bacteria were isolated from 92 specimens (90.19%), while the remaining 10 (9.80%) showed no bacterial growth. kidney failure were most prevalent in the 50-59 age group, with 40 patients (39.21%) affected, and the least affected group was 10-19 years old. Molecular analysis revealed the presence of key virulence genes, including *SurA1*, *OmpA* and *bas D* . The *SurA1* gene was detected in all isolates, suggesting its critical role in bacterial survival and pathogenicity. In contrast, the *OmpA* gene appeared in fewer isolates, indicating genetic diversity among strains. **Conclusion:** These findings emphasize the potential of *SurA1* as a reliable virulence marker in clinical isolates of *A. baumannii* and *bas D* gene found five isolates out of ten isolates.

Keywords: kidney failure, *Acinetobacter baumannii*, *Omp A* gene, *Sur A1* gene

1. Introduction

Chronic kidney disease (CKD) is defined as a gradual decrease of kidney function over months or years, which is frequently caused by long-term diseases such as high blood pressure or diabetes [1]. It is estimated that millions of people across the globe have CKD, and amongst these are those who suffer from increased morbidity as well as mortality [2].

The increasing prevalence of acute kidney injury (AKI) among hospitalized and Intensive care unit (ICU) patients presents a significant challenge, resulting in prolonged hospital stays, a greater demand for renal replacement therapy (RRT), elevated mortality rates, and long-term morbidity [3].

Dialysis patients also are vulnerable to colonization and infection by multidrug-resistant organisms, including

Acinetobacter baumannii. In this case, susceptibility is heightened due to the two factors of repeated vascular access and a compromised immune system [4]. *A. baumannii* is a Gram-negative bacterium in hospital-acquired infections, particularly affecting patients with impaired health, such as those with kidney failure [5].

Understanding the hazards associated with *A. baumannii* infections in renal failure patients emphasizes the significance of strict infection control and adequate antibiotic selection [6]. Outer membrane protein A (*OmpA*) has been identified as a virulence factor for *A. baumannii*. It promotes adhesion to epithelial cells, allowing for invasion and contributing to the bacterium's pathogenicity [7].

OmpA can induce apoptosis in host cells by targeting mitochondria, leading to cell death [8]. The surface antigen protein 1 (*SurA1*) had a role in the pathogenicity of *A. baumannii* infections, as demonstrated by the *surA1* gene knock-out approach. As a result, a link was discovered between *SurA1* protein shortage and improved bactericidal activity in human sera [9]. The *surA* gene encodes surfactant synthesis protein A, a protein involved in the synthesis of surfactants. These surface-active molecules enhance bacterial adhesion and facilitate biofilm formation. *SurA* plays a crucial role in the initial stages of biofilm development by facilitating cell attachment to surfaces [10].

Biosynthesis of aerobactin system gene D (*BasD*), have been identified in *A. baumannii* and are believed to contribute to its virulence and antibiotic resistance [11].

The *BasD* gene codes for an enzyme involved in the production of acinetobactin, the aforementioned siderophore. Acinetobactin plays a vital role in the acquisition of iron. The activity of *BasD* is essential for the bacterium's ability to produce acinetobactin, thereby enhancing its virulence and resistance [12]. *BasD* play critical roles in *A. baumannii* infection and apoptosis in epithelial cells [13]. This research aimed isolated of genes that caused pathogenicity and antibiotic resistance in *a.baumannii* isolated from kidney failure patients.

2. Materials and Methods

Collection of specimens

In this research 102 specimens were collected from kidney failure patients in AL- Najaf Governorate. Iraq,

from (September to November 2024).the specimens were urine and swaps from oral cavity collected with the assistance of a physician.

Isolation and diagnosis of bacteria

The types of gram-negative bacteria identified using biochemical testing and vitek 2 system . Specimens were swaps from oral cavity and urine were taking from patients. All specimens were grown on MacConkey agar, using Selective media for Acinetobacter, CHROMagar Acinetobacter and Leeds Acinetobacter Medium.

Extraction of DNA

Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's protocol with slight modifications. Briefly, 1 mL of overnight culture was centrifuged and the pellet resuspended in 480 µL of 50 mM EDTA. After addition of lytic enzyme, the sample was incubated and centrifuged. Nuclei Lysis Solution (600 µL) was added, mixed, and incubated for 5 minutes, followed by RNase treatment. Protein Precipitation Solution was added, vortexed, incubated on ice, and centrifuged. The supernatant was transferred to a tube with isopropanol, mixed, and centrifuged. The pellet was washed with 70% ethanol, air-dried, and rehydrated in 100 µL of Rehydration Solution at 65 °C for 1 hour or at 4 °C overnight.

Identification of genes

PCR testing was performed to detect antibiotic resistance genes and virulence factors of *A. baumannii*. In monotypes in order to amplify different parts of genes. The *OmpA*, *surA1* and *bas d* from bacteria were selected to be amplified separately using the single-strand PCR technique used in this study.

Table (1) Primers (Macrogen, Europe) used in this study

Primer Type	Primer Sequence (5'-3')	Amplicon size (bp)	Reference
<i>OmpA</i>	F: GTTAAAGGCGACGTA GACG R: CCAGTGTTATCTGTGT GACC	578	[14]
<i>surA1</i>	F: CAATTGGTAGCTGGC GATCA R:	241	[15]

	TTAGGCGGGACTCAG CTTTT		
<i>bas D</i>	F: CTCTTGCATGGCAAC ACCAC R: CCAACGAGACCGCTT ATGGT	868	[15]

Statistical analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Chi-square test was used to study association between any two categorical variables. The level of significance was considered at P-value of less 0.05 and highly significant level at 0.01 or less [16].

3. Results and Discussion

The types of bacteria and age

The results of this research appeared that 102 specimens were collected from patients suffering from kidney failure, 92 (90.19 %) specimens had been given positive growth of gram negative bacteria while 10 (9.80 %) of specimens showed no growth of gram negative bacteria, the results of the gram-negative isolates indicated that *Acinetobacter baumannii* was the most dominate bacteria with 20(19.6%) isolates followed by *Klebsiella pneumonia* 15(14.7%) isolates, and the lowest number is *Aeromonas hydrophila* 2(1.96%) as shown in table (2), kidney failure were most prevalent in the 50-59 age group, with 40 patients (39.21%) affected, and the least affected group was 10-19 years old, with 3 patients (2.94%) as shown in table (3). There is an agreement between the current findings and previous research [17], who found that chronic kidney disease was more prevalent among individuals in their fifth decade of life.

Table 2: Percentages of bacterial species of renal failure infection.

Characteristic	Percentages%	P value
<i>Bacterial species</i>		
<i>Acinetobacter baumannii</i>	20 (19.60)	
<i>Aeromonas hydrophila</i>	2 (1.96)	
<i>E. coli</i>	13 (12.74)	

<i>Enterobacter cloacae</i>	10 (9.80)	0.001 ¥ S
<i>Klebsiella Pneumonia</i>	15 (14.70)	
<i>Proteus mirabilis</i>	8 (7.84)	
<i>Salmonella</i>	4 (3.92)	
Total	72	

n: number of cases; ¥: Chi-square test; S: significant at $p > 0.05$

Table 3: Comparison of frequency distribution of age groups of patients with renal failure infection.

Age (years)	Renal failure infection	P
	Number (percentage %)	
10-19	3 (2.94)	0.001 ¥ S
20-29	5 (4.90)	
30-39	6 (5.88)	
40-49	15 (14.70)	
50-59	40 (39.21)	
60-69	29 (28.43)	
70-79	4 (3.92)	

SD: standard deviation; n: number of cases; ¥: chi-square test; S: significant at $P < 0.05$.

Current research closely matches a local research conducted in Kirkuk, Iraq, which reported that 35.0% of dialysis patients were in the 50–59 age group , this similarity reinforces the observation that middle-aged adults are at greater risk of developing chronic kidney disease in the Iraqi population [18].

Similarly, a previous study by [19], reported that 50 out of 110 urine samples (45.5%) collected from patients with chronic renal failure showed bacterial growth. Among these, Gram-negative bacteria were the predominant isolates, accounting for 68% of the total bacterial growth, these findings indicate a consistent trend across studies, emphasizing the significant role of Gram-negative bacteria in infections among kidney failure patients.

Virulence Factors of Acinetobacter baumannii

Detection of antibiotic resistance genes (Omp A , Sur A1 and bas D), the amplification results of PCR study for Omp A (578bp) , Sur A1 (241bp) and bas D (868) , revealed that (100%) of *A. baumannii* isolate gave positive for Sur A1. As show figure (1), and as show figure (2) (40%) of *A. baumannii* isolates gave positive result for Omp A gene, and figure (3) showed that 5 isolates (50%) of *A. baumannii* were possessed basD gene.

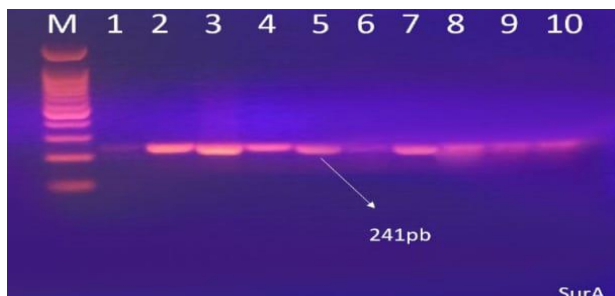


Fig 1: The PCR product of *SurA* gene in 1% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 μ L of PCR product loaded in each well. Lane M: DNA Ladder (3000bp), PCR product (positive case band 241 bp).

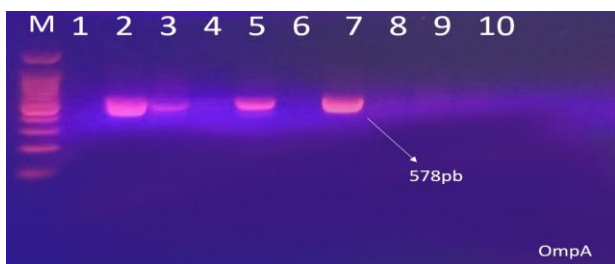


Fig 2: The PCR product of *OmpA* gene in 1% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 μ L of PCR product loaded in each well. Lane M: DNA Ladder (3000bp), PCR product (positive case band 578 pb).

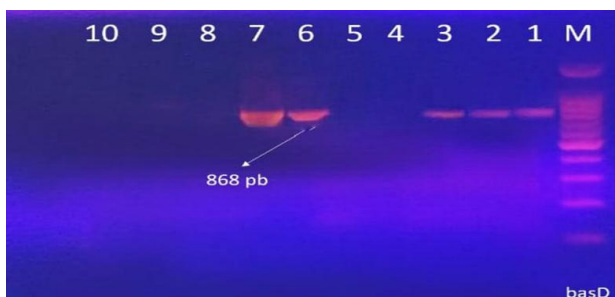


Fig 3: The PCR product of *basD* gene in 1% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 μ L of PCR product loaded in each well. Lane M: DNA

Ladder (3000bp), CR product (positive case band 868 bp).

Current results in agreement with findings [20] which indicated the changes in the expression level of *SurA1* may occur with alterations in antibiotic resistance and virulence in bacteria. The previous research aimed to determine the relationship between *SurA1* and *A. baumannii* virulence and fitness.

The outer membrane (OM) of Gram-negative bacteria is a unique architecture that acts as a defensive barrier to toxic molecules. It is composed of phospholipids, lipopolysaccharide (LPS), outer membrane β -barrel proteins (OMP), and lipoproteins [21]. The outer membrane protein A (OmpA) is one of the components of OMPs of several Gram-negative bacilli. It is a key virulence factor that mediates bacterial biofilm formation, eukaryotic cell infection, antibiotic resistance, and immune regulation [22].

The results agreement with findings [23] that indicated they play an essential role in maintaining the bacterial structure, substance transport, cell surface recognition, signal transduction, and pathogenicity. However, they are also involved in physiological functions such as bacterial infection, adhesion, inflammation, activating the host to produce immune protection, and involvement in drug resistance.

BasD enzyme is responsible for the production of acinetobactin. The *bas D* contribute to the bacterium ability to survive and cause persistent infections [24].

In previous research the results of PCR and gel electrophoresis showed that in 82% of the MDR strains, both *BauA* and *BasD* genes were successfully detected [25].

Conclusion

Out of 105 specimens 92 (90.19 %) specimens had been given positive growth of gram negative bacteria while 10(9.80%) of specimens showed no growth of gram negative bacteria. The age groups from (50 to 59) years are the most susceptible to kidney failure, with a number of 40 ,compared to the age groups from (10 to 19) years, which had the lowest incidence of infection.

The results of the gram-negative isolates indicated that *A. baumannii* was the most dominate bacteria.

Among the virulence factors of *A. baumannii*, the most frequently isolated bacterium from kidney failure patients, the *SurA1* gene was detected in all isolates, while the *OmpA* gene was also present but with a lower

frequency. This suggests that *SurA1* may play a more prominent role in the pathogenicity of the isolates..

Funding Information

No conflict of interest is present

Ethics

The review was endorsed by the ethical committee of the faculty of science university of Kufa, al-Najaf city, Iraq.

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