

Molecular characterization of *Acinetobacter baumannii* isolated from patients with respiratory tract infections

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Abstract:

This study aimed for molecular characterization of *Acinetobacter baumannii* isolated from patients with respiratory tract infections from clinical specimens includes , sputum , lower respiratory secretion of patients admitted to Medical Teaching City, and Al-Yarmouk Teaching Hospital in Baghdad, Al-Hussein teaching hospital in Karbala ,with study the rate of biofilm formation in *Acinetobacter baumannii*, in addition to investigate the to determine the frequency of responsible virulence genes. during a period from the first of October 2020 to the end of May 2021. The predominant symptom was Chest pain encountered in patients (22.45%). Ranking in the lowest order Smoking is which was seen in patients (9.80%). In present study, out of 16 isolates there were (50%) possessed RecA gene, Sur A gene (62.50%), PAI gene (56.20%) ,Fim H gene (81.20%) respectively. By PCR amplification, the present results showed that, the amplification products of sequences of RecA gene, ITS gene ,Sur A gene PAI gene and Fim H gene in chromosomal DNA were of size 425 bp 208bp, 241bp, 930bp and 508bp respectively. In the current study, the distribution of genes in *Acinetobacter baumannii* clinical isolates showed that, , the highly percentage gene Fim H was identified in all isolates 20(80%), followed SurA 16(64%), ITS 14(56%) ,RecA Gene and PAI 12(48%) genes respectively.

In Conclusion, In Iraqi hospitals, *Acinetobacter baumannii* was the most common bacterium found in patients with respiratory illnesses.

Keywords: *Acinetobacter baumannii*, PCR, Genes, respiratory.

Introduction:

In healthy persons, *Acinetobacter* genus members are thought to have a limited ability to cause disease. On the other hand, certain species, particularly *A. baumannii*, may cause life-

threatening infections in people with impaired immune systems. A typical opportunistic pathogen, it is regarded.)**1(**

UTI, bacteremia, wound and burn infections, meningitis and most crucially nosocomial pneumonia (especially in ventilated patients) are all manifestations of *A. baumannii* infections (2). *Pseudomonas septica* and *Klebsiella pneumoniae* are the most significant Gram-negative nosocomial bacteria at this point in time.)**3(**

Acinetobacter bacteremia is linked to a significant death rate. Infections in the urinary tract, surgical wounds, burns, and infected intravenous catheters, tubes, and canules are the most frequent causes of bacteremia, whereas in 21–70 percent of the cases, the source is unclear (4). Compared to other pathogens, *A. baumannii* is more often detected in mixed blood infections (5) and may be present in 10-15 percent of all mixed infections (6(

An rise in the incidence of *Acinetobacter*-caused pneumonia occurred between 1976 and 1990, from less than one percent to six percent (7), *Acinetobacter*, along with *P. aeruginosa*, *S. aureus*, and *Enterobacteriaceae*, is one of the most common causes of ventilator-associated pneumonia (VAP) at the present moment.)**7,8(**

Neurosurgery, head injury, acute respiratory distress syndrome, prior antibiotic treatment, aspiration, inadequacies in the implementation of infection control regulations, and a protracted hospital stay all contributed to *Acinetobacter* related nosocomial pneumonia.)**7(**

In homologous recombination, the enzyme RecA is a key player, and it also plays an important part in SOS mutagenesis. This multidrug-resistant bacteria *Acinetobacter baumannii* is responsible for global nosocomial infections has distinct DNA repair responses from other bacterial species, including those of *Acinetobacter* spp. An *A. baumannii* RecA mutant was created to test the function of RecA.

Several studies have indicated that the *fimA* gene encodes the large secondary unit while the *fimF* and *fimG* genes encode the small subunits and the *fimH* gene encodes the top of the cilia that are sensitive to the manus and the *fimC* gene encodes the attached protein that helps the *fim* protein pass through the Periplasms and *fimD* encodes the outer membrane proteins and *fimI* encodes For the structural of the grass and molecular weight of *fimH* gene was 508 bp . The *fimH* gene is an important virulence agent for bacteria. Which encodes the Type 1 fimbriae, that helps bacteria bind to the surface of host cells and then cause injury **(10).**

This study aimed for molecular characterization of *Acinetobacter baumannii* isolated from patients with respiratory tract infections.

Materials and Methods:

This study ran from October 1, 2020, through May 31, 2021. More than a hundred clinical specimens from patients at the Medical Teaching City, Al-Yarmouk Teaching Hospital in Baghdad, Al-Hussein Teaching Hospital in Karbala were obtained for this study.

After sterilization at 121°C (15 lb/in²) for 15 minutes and incubation at 37°C for 24 hours to establish sterility, the media were stored at (4°C) until they were utilized, per the manufacturer's instructions.

All samples were grown in aerobic conditions for 24 hours on blood agar and MacConkey agar at 37 degrees Celsius. Prior to performing a biochemical identification according to Cheesbrough's standard procedures, the isolated bacteria were first identified using morphological characteristics of the colonies such as size, shape, color, odor, and pigment production. A single colony was then picked with a sterile loop to prepare a pure subculture on nutrient agar in broth (11).

DNA extraction

Nano drop .Geneaid Extraction was used to extract genomic DNA from bacterial growth instruments were used to measure DNA concentration and purity per the manufacturer's instructions; 3 l were aspirated using a special tip (Aeroject tips 10 l) and placed in the machine's socket; DNA concentration and purity were then measured using the refractive index at wave lengths of 260nm and 280nm. The OD 260nm/OD 280nm ratio was used to determine the purity, whereas the OD260nm was used to determine the DNA content. DNA purity was defined as a ratio of less than 1.8, indicating a minimal level of protein contamination

Primers

Conventional PCR was used to detect the presence of specific gene for diagnosis *Acinetobacter baumannii*. Primers sequences for genes were as follow:

Table 1. Primers sequences.

Primer Name	Nucleotide sequences (5' → 3')	Products bp	References
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RecA gene	F	CCTGAATCTTCTGGTAAAAC GTTTCTGGGCTGCCAAACATTAC	425	Nowak et al.,2009 (122)
	R			
Sur A gene	F	CAATTGGTAGCTGGCGATCA TTAGGCGGGACTCAGCTTTT	241	Liu et al., 2016 (124)
	R			
PAI gene	F	GGACATCCTGTTACAGCGCGCA TCGCCACCAATCACAGCCGAAC	930	Johnson et al., 2003 (125)
	R			
fim H gene	F	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	Johnson & Stell (2000) (126)
	R			

To amplify these RecA gene of *Acinetobacter baumannii* isolates, the mixture of conventional PCR working solution was prepared as follow (Table 2):

Table 2. PCR solution concentrations and volume

Component	Volume µl
Master mix	12.5µl
Primer F.	2.0 µl
Primer R.	2.0 µl

DNA template	3 µl
Deionized Distilled water	5.5 µl
Total Volume	25 µl

Table 3 shows the PCR cycle program settings that were employed in this experiment to identify genes.

Table 3 PCR program for detection of genes amplification by thermal cycler.

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55, 56 or 58	00:30	
Extension	72	00:30	
Final extension	72	07:00	1

PCR products were resolved using agarose at a concentration of 1.5%. It was shot utilizing a gel imaging system after electrophoresis using UV trans-illuminator and UV tran-illuminator.

Statistical methods

The Statistical Analysis was done by using SPSS 20.

Results:

Table (4) shows the clinical manifestations of respiratory infections patients subjected to the present study. The predominant symptom was Chest pain encountered in patients (22.45%). Ranking in the lowest order Smoking is which was seen in patients (9.80%).

Statistically, no significant Symptoms differences observed in our study.

Table(4): Clinical manifestation of respiratory patients.

	Acinetobacter baumannii
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		Positive		Negative		Total	P value
Smoking	Yes	5	9.80%	46	90.20%	51	0.001**
	No	20	40.82%	29	59.18%	49	
Chest pain	Yes	11	22.45%	38	77.55%	49	0.647 ^{NS}
	No	14	27.45%	37	72.55%	51	
Fever	Yes	10	16.13%	52	83.87%	62	0.016*
	No	15	39.47%	23	60.53%	38	
Chills	Yes	3	20.00%	12	80.00%	15	0.755 ^{NS}
	No	22	25.88%	63	74.12%	85	
Trouble breathing	Yes	8	15.69%	43	84.31%	51	0.038*
	No	17	34.69%	32	65.31%	49	

NS: none statistical significance (p>0.05)

*: Statistical significance (p≤0.05)

**: High statistical significance (p≤0.001)

Detection of genes (RecA, Sur A ,PAI, and Fim H) was performed by theconventional PCR technique.

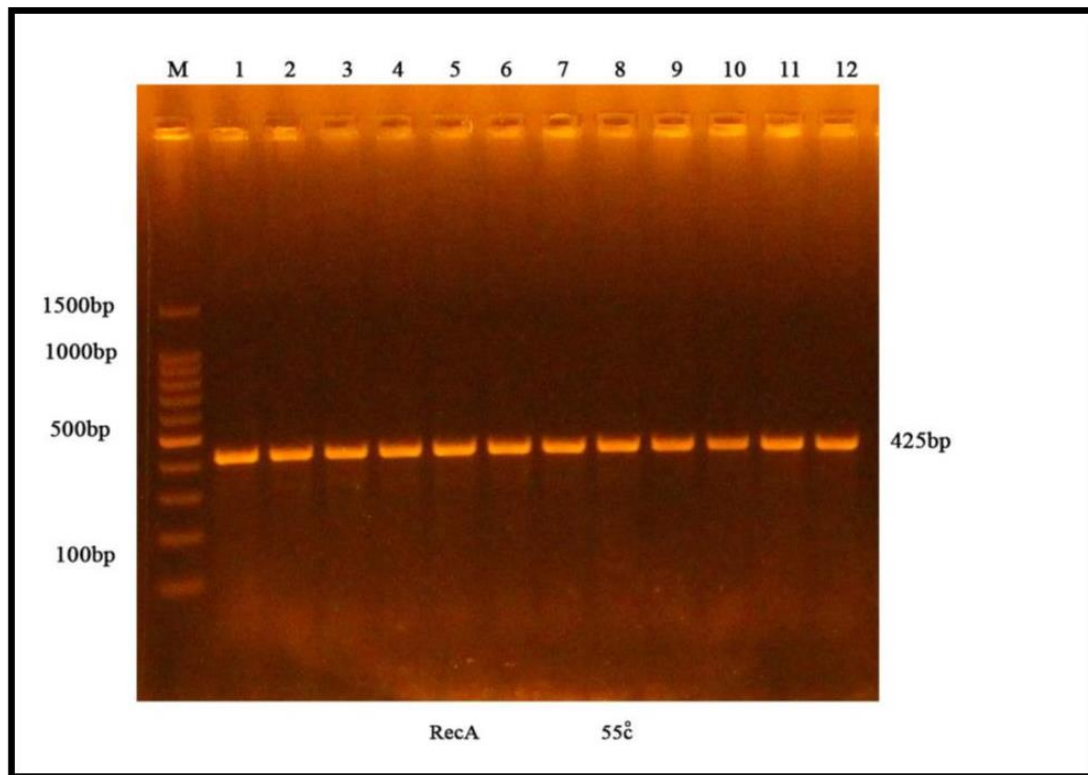


Figure (1):The amplification results of RecA primers in *Acinetobacter baumannii* species fractionated on (1.5% agarose, 100v/mAmp for 75min) stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 425bp PCR products.

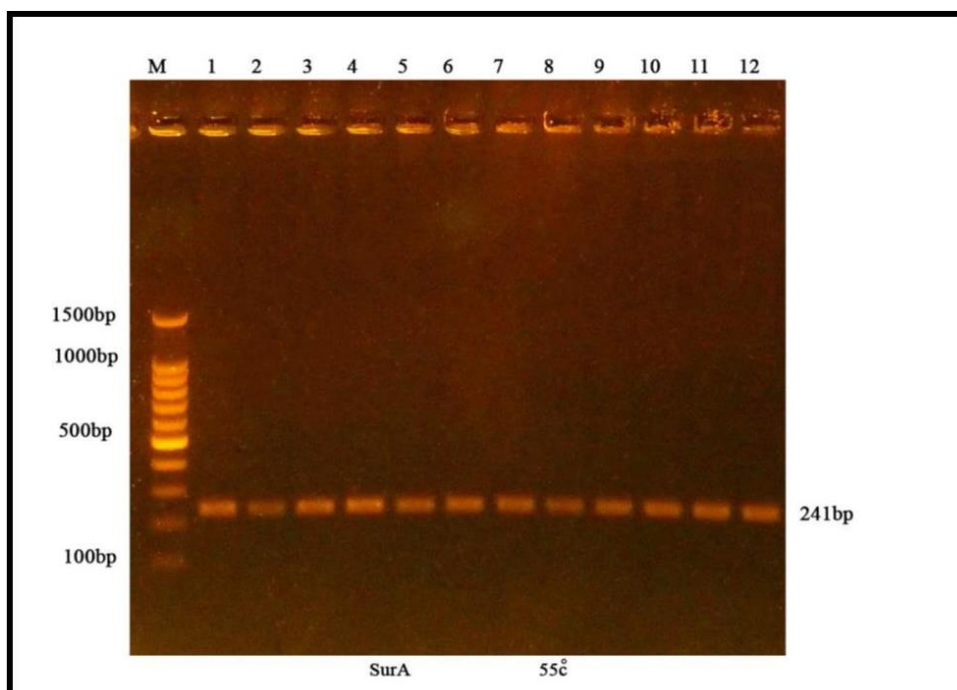


Figure (2):The amplification results of SurA primers in *Acinetobacterbaumanniis* pecies fractionated on(1.5% agarose,100v/mAmp for 75min) stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 241bp PCR products

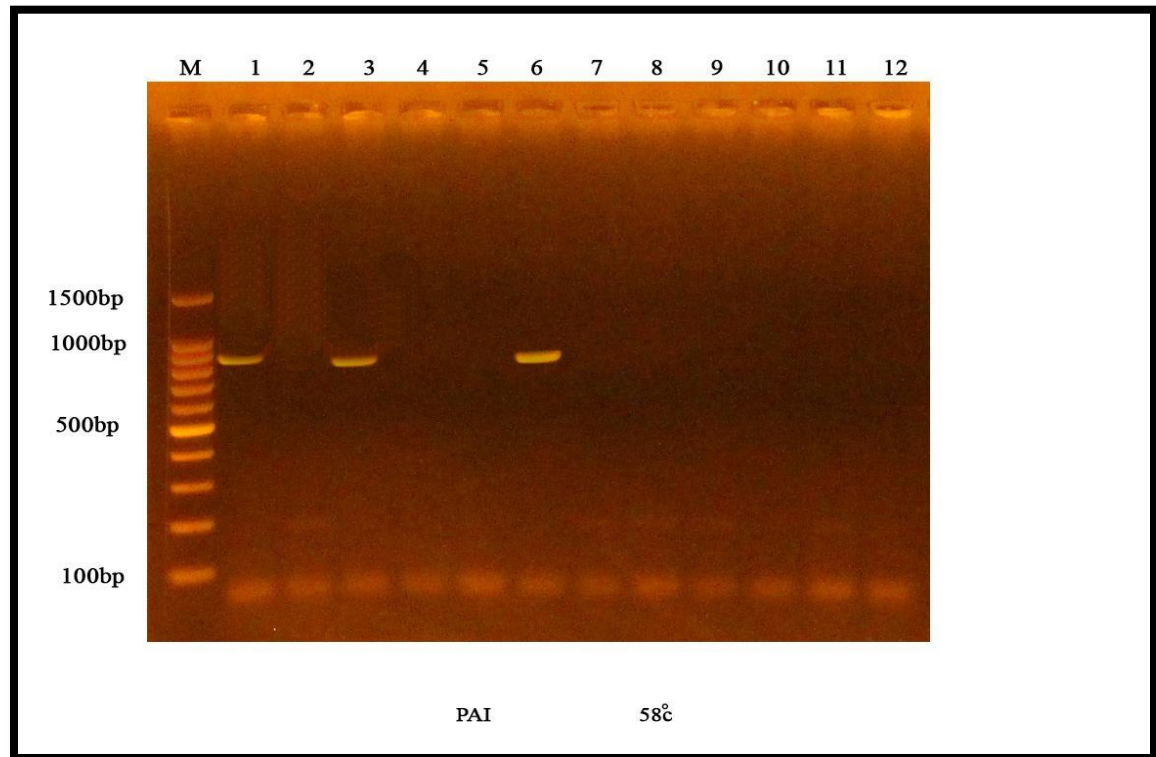


Figure (3): The amplification results of PAI primers in *Acinetobacterbaumanniis* pecies fractionated on(1.5% agarose,100v/mAmp for 75min) stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 930bp PCR products

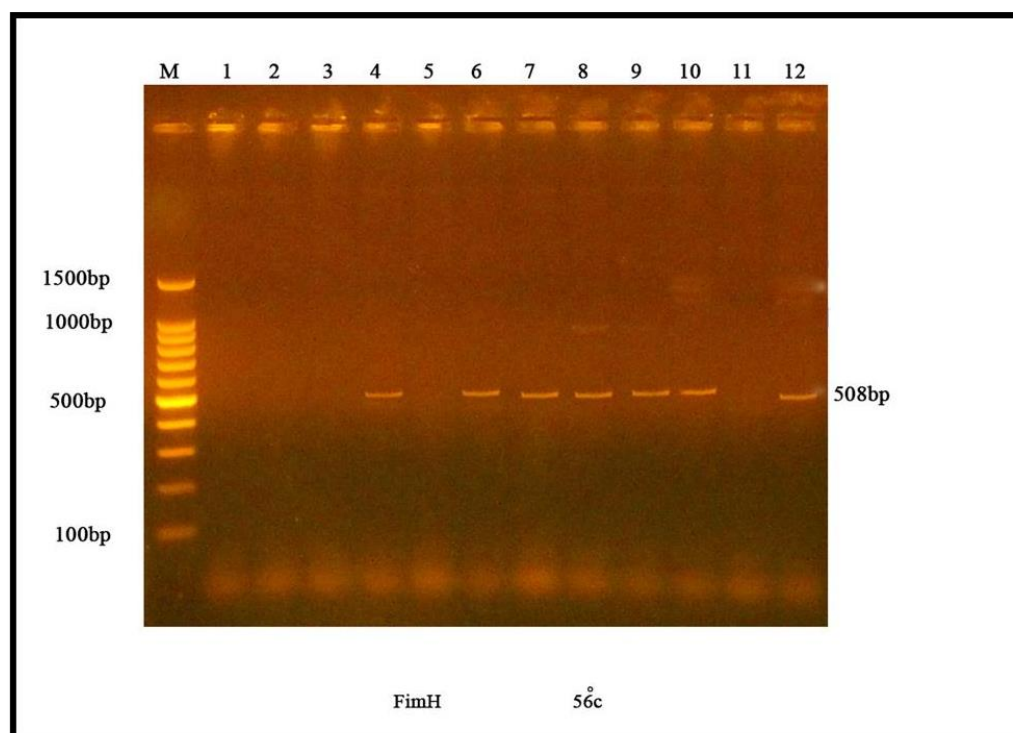


Figure (4): The amplification results of FimH primers in *Acinetobacterbaumanniis* species fractionated on(1.5% agarose,100v/mAmp for 75min) stained with Eth.Br. M: 100bp ladder marker. Lanes 1-19 resemble 508bp PCR products

Table 5: Distribution of genes in *A. baumannii* clinical isolates

Genes	Positive	Percent	Negative	Percent
RecA Gene	12	48	13	52
ITS	14	56	11	44
Sur A	16	64	9	36
PAI	12	48	13	52
Fim H	20	80	5	20

The results from table (5) revealed that all examined isolates of *A. baumannii* had genes with a varying presence. However, the highly percentage gene Fim H was identified in all isolates 20(80%), followed SurA 16(64%), RecA Gene and PAI 12(48%) genes respectively.

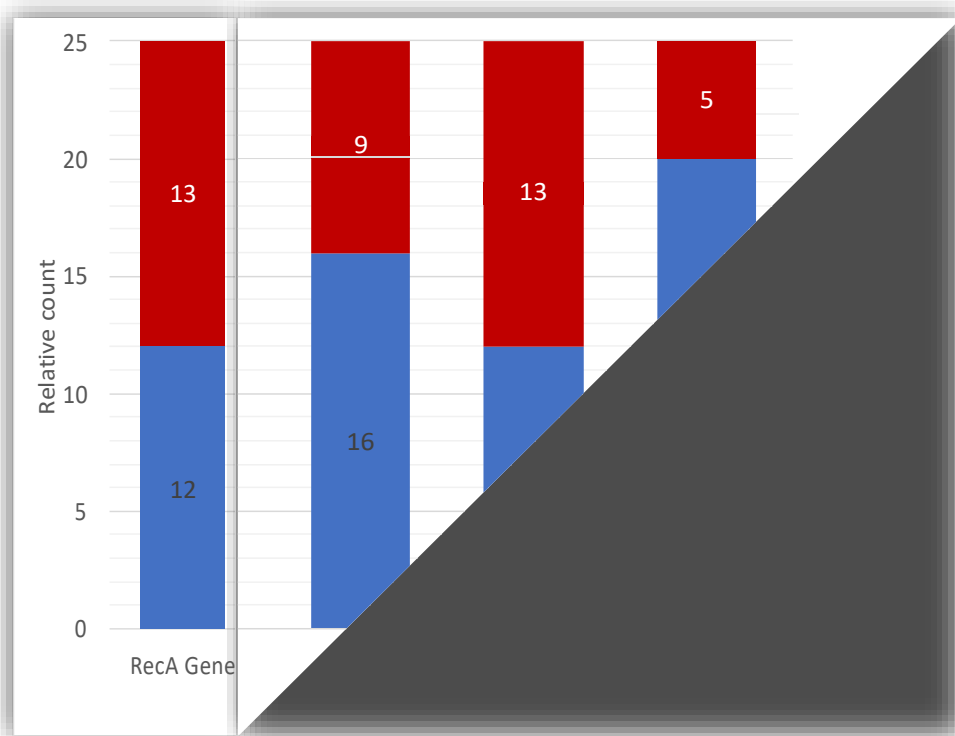


Figure 5: the prevalence of genes in *Acinetobacter baumannii* isolates

Discussion:

Patients and Microbial identification

Acinetobacter baumannii was found in 25 percent of Iraq samples compared to other microorganisms with various illnesses, according to this research. *A.baumannii* was shown to be more prevalent than other bacteria in a research done in Baghdad by AL-Kadhmi, N.A.2018(12). While in Egypt, *A.baumannii* was found in 62.5 percent of samples (13). The Riyadh Military Hospital research also found that *A.baumannii* had a higher proportion (40.9 percent) than other bacteria (14). However, the findings of the present investigation were at odds with those of an earlier study done in Baghdad by AL-Kadhmi, N.A 2016(15), which found that *S.aureus* (30 percent) and *A.baumannii* (10.6 percent) were the most prevalent pathogens found in Iraqi patients with wound infections. In this study, the researchers used a different set of samples than in previous investigations. *A. baumannii* infection was shown to be related with higher immediate temperature values in addition to climatic variability (16). Patients who live in tropical climates are also at risk for *Acinetobacter baumannii* infection, according to Sunenshine R.H. 2007(17). Some 26 (72.2 percent) *A. baumannii* isolates came from respiratory tract infections, according to another research (18). Males had a 27.59 percent isolate rate, while females had a 21.43 percent isolate rate, according to

this research. According to Iran (19), (67%) of *A. baumannii* was found in men, and (32.6%) in females, in this research. In addition, a research in Palestine(20) found that 62.5 percent of males and 37.5% of females were found to have the disease. *A. baumannii* was found in 42% fewer men than females in the present investigation, which contrasts with a study done in Iraq (21) that found (58 percent). Males were found to have a greater proportion of isolates (70.96 percent) than females (29.03 percent). According to the Egyptian research (22,23), 67.5 percent of men, 51.5 percent of females, and 76.0 percent of all males were found, respectively. Al Hassan A.R. 2012 (24), on the other hand, reported that 50.9 percent of females outnumbered men 49.1 percent. Due to males' greater exposure to the conflicts, the proportion of *Acinetobacter baumannii* infection in males was higher than in females; in addition, the research in Iraq and Kuwait found that soil samples collected from Iraq and Kuwait were highly contaminated with *Acinetobacter baumannii* (25).

This research found that the age group (>45 years) had the greatest proportion of isolates (25.45%), while the age group (< 45 years) had the lowest percentage (24.44%). Due to the contamination of incubators with *Acinetobacter baumannii*, neonatal infections are the most common (26,27). The lack of maternal IgM transport, which acts as heat-stable opsonins, increases the newborn's vulnerability to Gram-negative bacteria infection (28). The mother's immune system also plays a role in the development of neonatal sepsis.)29(

In contrast to a Saudi Arabian investigation (30) of samples from acute care units, this study found that the largest proportion of isolates was in the 60-plus age group. We differed with the results of a similar investigation in Egypt (31) that found a 47.5 percent prevalence of isolates among those ages 30 to 39, whereas the lowest proportion of isolates among those ages 40 to 39 was only 10%. (20-39year). According to (32), the biggest proportion of isolates (47.5 percent) was found in the 40-59-year-old age group, while the lowest amount was (10 percent) found in the younger age groups (20-39 years). Because individuals in the 20-39-year-old age range are immunocompetent.

These infections are very rare but when they do occur (e.g. in the respiratory tract, the urine, or the blood), they often include organ systems with a high fluid content (e.g. the respiratory tract, the urinary tract, or blood), as well as enzymes that may damage blood vessels lipids (33). (34). During *Acinetobacter* septicemia, endotoxin generation in the body is likely to be the cause of illness symptoms (35). *A. baumannii* infection may cause a variety of unusual symptoms in people with chronic obstructive pulmonary disease who live in tropical settings (36).

The current results showed that revealed that all examined isolates of *A.baumannii* had genes with a varying presence However, the highly percentage gene Fim H was identified in all isolates 20(80%),followed SurA 16(64%), ITS 14(56%) ,RecA Gene and PAI 12(48%)genes respectively.

The current result showed that (80%), percentage of *Acinetobacter baumannii* isolates have Fim H gene,Several studies were varied with present study results.In study from Iraq **(37)** they found that the fimH gene was present in (20) isolates (50%) of *Acinetobacter baumannii* , while other study in Iran reported that all isolates of *Acinetobacter baumannii* (100%) had fimH gene**(38)**. The result was closely correlated with the findings of the researchers **(39)**.Who found 70% of *A. baumannii* isolates possessed the fimH gene with molecular weight of was 508 bp and the results were agreed with researchers Momtaz et al. **(40)**. Who found that 90 isolates (47.38%) of *A. baumannii* had this gene that isolate from hospital infection.

The SurA gene was found in 64% of *Acinetobacter baumannii* isolates in the present study. Those findings were in line with those of an Iranian research from 2015 (41) which revealed that (60%) of *A.baumannii* isolates tested positive for the SurA gene, while another Iranian study from 2016 found that (66%) of isolates tested positive for the SurA gene. 49 of the isolates tested had the SurA gene (98 percent), according to a research in Iran in 2021.)43(

Acinetobacter baumannii isolates with RecA Gene and PAI genes (48 percent) were found in the present study.

Current research shows consistency with an earlier Egyptian study (200). According to Al-Harmoosh.RA. 2015, the RecA and PAI genes were discovered in 20% and 40% of *A.baumannii*, respectively, in Iraq's first investigation (44). However, a different investigation found that all *Acinetobacter baumannii* isolates possessed the RecA gene. Almost all *Acinetobacter baumannii* isolates (46.6 percent) possessed the PAI gene, which is consistent with the current findings (45).

Conclusion:

In Iraqi hospitals, *Acinetobacter baumannii* was the most common bacterium found in patients with respiratory illnesses.

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