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Research Article

Bacteriological Study of *Acinetobacter baumannii* Isolated from Patients with Blood Infections in Al-Najaf City

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Abstract: From November 2022 to February 2023, a total of 50 clinical specimens were taken from individuals with blood infections. Patients who visited AL-Sadder Medical City and AL-Hakem General Hospital during the study time provided these specimens. All samples were grown on blood agar and MacConkey agar plates and they were all then incubated for 18-24 hrs at 37°C under aerobic conditions. Among a sample of 50 patients, an equal number of 25 (50%) were identified as female and 25 (50%) as male. Various morphological, physiological and biochemical examinations were conducted in order to ascertain the identification of bacterial isolates. The findings of the study indicate that *A. baumannii* accounted for 8 isolates 16% of the total isolates, whereas the remaining isolates were identified as *E. coli* and *P. aueroginosa*. The study involved a set of 8 isolates of *A. baumannii*, which were identified based on their morphological, cultural and biochemical characteristics. The final confirmation of their identification was carried out using the Vitek-2 compact system, which revealed that only 6 (75%) of the isolates were indeed diagnosed as *A. baumannii*. The research examined the genes responsible for encoding virulence factors in *A. baumannii*, which are crucial for its pathogenicity, including *pap* and *FimA*. The aforementioned genes play a significant role in the processes of invasion and adhesion. The results of the research demonstrated that *A. baumannii* virulence factors are crucial to the pathogenesis of the bacteria.

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INTRODUCTION

So basically, *Acinetobacter baumannii* is a type of bacteria that doesn't like glucose and is not very friendly. It can make people sick, especially if they're already not feeling great or have had some medical procedures done. It's been causing more and more infections in hospitals lately, especially in the ICU[1].

Acinetobacter baumannii is like the most important and common *Acinetobacter* species that doctors deal with Endo *et al*.[2]. Yo, in the past 20 years, *A. baumannii* has become a big deal in the medical world because it's really good at causing infections and can resist almost all antibiotics out there[3].

Acinetobacter baumannii ability to stick to surfaces and chill there as biofilms might be a big reason why it's so dang harmful. To make a strong biofilm on both living and non-living surfaces, you gotta have pili, a biofilm protein and be able to resist antibiotics. So basically, this whole thing relies on how certain functions control gene expression and can tell if there's enough nutrients for the cells[4].

So basically, *A. baumannii* has these things called virulence factors that help it stick to human cells. One of them is a capsule made of sugars and the other is called fimbriae[5].

So basically, this bacteria called *A. baumannii* has these tiny little hair-like things called pili that are made up of protein subunits called fimbrins or pilins. They're like 1-3 µm long and 2-8 nm wide. So, basically, Pili are super important for bacteria to stick to stuff and make biofilm that can cause infections in humans[6].

Acinetobacter baumannii germs are tough to kill with drugs and can hang around in hospitals for a long time, making them hard to get rid of Liakopoulos *et al*.[7].

MATERIALS AND METHODS

Specimens collection: Clinical samples from 50 patients with blood infections were obtained between November 2022 to February 2023. During the time span under review, these samples were collected from patients at AL-Sadder Medical City and AL-Hakem General Hospital. All samples were grown on MacConkey agar plates and blood agar, then put in an aerobic incubator at 37 degrees Celsius for 18-24 hr[8].

Isolation and identification of *A. baumannii*: MacConkey agar and blood agar were used as separation media for all samples. Agar plates were incubated at 37 degrees Celsius for 24 hrs to determine if they contained pure culture colonies. The VITEK-2 compact system was then used to conduct a biochemical test, which verified the presence of the bacteria.

Molecular study of *A. baumannii*

DNA Isolation and analysis: A industrial DNA extraction device was used to obtain the genomic DNA. (Genomic DNA promega Kit).

Genes encoding virulence factors, such as *pap* and *FimA*, were identified using a polymerase chain reaction (PCR) test.

All of the primers used in this research were made by the Bioneer business in Korea (Table 1)[9].

PCR Program: It is listed in Table 2.

Agarose preparation: Agarose preparation was accomplished by Mishra *et al*.[10].

RESULTS

Bacterial isolation: Patients with blood infections were recruited from November 2022-February 2023 and a total of 50 clinical tissues were obtained. Samples were taken from patients at AL-Sadder Medical City and AL-Hakem General Hospital during the study's time frame. After being placed in an aerobic 37°C incubator for 18-24 hrs, all samples were grown on MacConkey agar plates and blood agar. About 25 of the 50 patients were feminine and 25 were male, as shown in Fig. 1.

Table 1: Primers used in this study

Primer types		Primer (5'-3')	Product size (bp)	References
pap	F	GACGGCTGTACTGCAGGGTGTGGCG	328 bp	Mishra *et al*.[10]
	R	ATATCCTTTCTGCAGGGATGCAATA		
FimA	F	GTTGTTCTGTCGGCTCTGTC	447 bp	Mishra *et al*.[10]
	R	ATGGTGTTGGTTCCGTTATTC		

Table 2: Thermocycler condition

		Temperature (°C)/time				
		Cycling conditions				
Gene name	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles no.
pap	94/5 min	94/45 sec	59.9/1 min	72/1 min	72/5 min	32
FimA	94/5 min	94/1 min	59.2/1 min	72/1 min	72/10 min	35

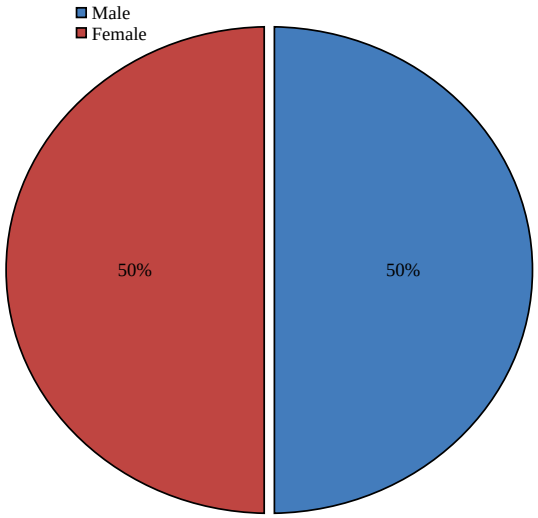

Fig. 1: Sex differences in the prevalence of infection

Infections caused by *Acinetobacter baumannii* have increased dramatically over the past three decades, making this bacterium a priority for medical attention[11].

As *A. baumannii* becomes increasingly isolated in clinical settings, it poses a significant threat to patients due to its ability to cause a wide variety of infections (including bacteremia, bacteriuria, pneumonia and wound, skin and soft-tissue infections) that are frequently fatal[12].

***Acinetobacter baumannii* isolation and identification:** Early on, we used cultural morphology, microscopic characteristics and biochemical tests to determine the identity of the bacterial isolates we acquired from clinical specimens. Culture-based confirmation of *A. baumannii* from those isolated strains. Colonies of *Acinetobacter baumannii* on blood agar were white/cream in color, smooth, circular and complete around the margins, while those on MacConky agar were pink in color, showed no pigmentation, were tiny in size and had regular edges. When viewed under the microscope, *A. baumannii* appears as gram-negative coccobacilli that are either singly or in pairs.

As a supplement to the primary identification of *A. baumannii* isolates, the outcomes of biochemical tests were examined (Table 3). Oxidase, indole, methyl red, Vogus-Proskuar, uricase, gelatin liquefaction and urease were all consistently present across all samples. Contrasted with the favorable results of catalase and citrate. They don't move around and don't process lactose. Regarding iron, sugar and triple sugar Hemolysis (hemolysis) produces an alkaline/alkaline crimson color without gas and no H_2S production.

Table 3: Traditional chemistry assays of *A. baumannii*

Biochemical test	Results
Catalase production	+
Citrate utilization	+
Growth at 44°C	+
Hemolysin production	-(γ hemolysis)
Indole production	-
Lactose fermentation	-
Motility	-
Oxidase production	-
Triple sugar iron agar	Alkaline slant/No change bottom, No gas, No H_2S
Urease production	-
Methyl-red	-
Voges-Proskauer	-
Gelatin Liquefaction	-
Capsule production	+

Acinetobacter baumannii colonies on MacConkey agar showed a pinkish tint due to non-lactose fermentation, no pigmentation, small size and regular edges, which is consistent with a diagnosis consistent with Constantiniu *et al*.[13], in other isolates, the colonies showed a purplish hue, which could pose a problem for organisms with lactose fermentation and was diagnosed as such by Lahiri *et al*.[14] and Forbes *et al*.[15]. A round, smooth, whitish or cream-colored object with clean, unbroken borders will form on blood agar when the correct[14].

The microscopic outcome of *A. baumannii*, wherein the cells look like coccobacilli and are set up singly or in pairs[16]. All of the biochemical assays for the isolates came back negative for oxidase, motility, indole production and urease production but positive for catalase and citrate consumption. H_2S levels dropped to zero and no gas was produced as the TSI turned alkaline[17].

All *A. baumannii* isolates tested positive for this method by causing a distinct light-brown discoloration of the surrounding blood agar (browning effect), whereas two other isolates (*Pseudomonas aeruginosa*) did not.

Since other membrane-bound GDH organisms, like *P. aeruginosa*, did not discolor the glucose-blood agar, we can conclude that the presence of soluble glucose dehydrogenase (GDH) is responsible for the discoloration (although this bacterium contains both soluble and membrane-bound GDH), which is consistent with the results of both of the previous studies[18].

Molecular Study (genotypic detection of *A. baumannii*): The ability to Adhesins and surface hydrophobicity are examples of a specific variables that play a part in bacterium adhesion. Additionally, the Chaperone usher pathway can be used to construct fimbriae, which are small, hair-like bacterial extensions. This process results in the production of two proteins. The first type of chaperone is a periplasmic one that aids in the

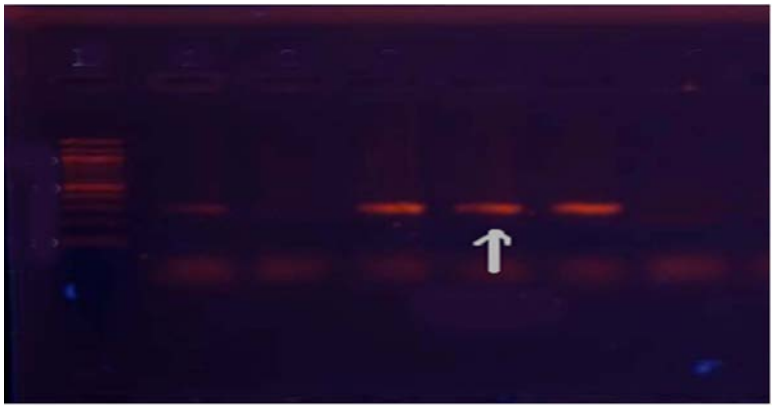

Fig. 2: Electrophoresis on an agarose gel marked with ethidium bromide for 1 hr at 80 volt cm^{-1} of PCR amplified products of *pap* gene primers yielding a 328 bp product

Fig. 3: Electrophoresis on an agarose gel labeled with ethidium bromide for 1 hr at 80 volt cm^{-1} of PCR amplified *FimA* gene fragments measuring 447 bp

assembly of pilus components. The second is an exterior membrane escort that helps the chaperone's components break apart, revealing their functional surface and propelling assembly into the pilus[19].

Bacterial adhesins are one component that can bond to and engage with many different surfaces. These adhesins can be either fimbrial or afimbrial, depending on the surface type they are designed to bind to. Many different types of bacteria exhibit a family of cell surface adhesins called fimbrial adhesins, which are able to identify and attach components of the extracellular matrix[20].

Six *Acinetobacter baumannii* samples were initially identified using a combination of physical, cultural and molecular characteristics; later, the identity was verified using the vitek-2 compact system. In agreement with Mishra *et al*.[10] finding that out of 3 *A. baumannii* isolates, (66.6%) was positive for *pap* and *FimA* as shown in Fig 2 and 3, the findings revealed that only 4 (66.6%) isolates was carrying *pap* and 4 (66.6%) isolates for *FimA*.

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