RESEARCH ARTICLE

Molecular Study of Virulence Genes in *Acinetobacter baumannii* Isolated from Specimens Clinical in Najaf Province of Iraq

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ABSTRACT

The objective of this research is to isolate and identify 24 *Acinetobacter baumannii* isolates from 251 clinical samples collected from patients in the Najaf Province. hospitals and visitors during a period from September, 2020 till the end of February /2021. The bacteria isolated from this specimens were obtained from patients infected with burns and wound infections, urinary tract infection (UTIs), bacteremia, respiratory tract infection (RTIs) and cerebrospinal fluid (CSF) were *A. baumannii* isolates isolated in high percentage in the wound and burn was 10 (41.6%), urine 7(29.1%),blood 4(16.6%), septum 2(8.3%) isolate and the later is CSF only 1(4.1%).

In this study all 24 of *A. baumannii* isolates were positive for biofilm formation, these results showed (62.5%) from all types of samples are strong, while about (25. %) from all types of samples are moderate biofilm and (12.5%) is weakly biofilm. and all bacterial isolates (24 isolate 100%) were able to resistant beta-lactamase antibiotics according to phenotypic detection. The *blaOXA-51* gene and the bap gene were found to be positive in all 24 (100%) isolates tested positive for PCR to detect several major virulence factors in *A. baumannii*.

Keywords: *Acinetobacter baumannii*, Infection, Virulence genes (*bap* and bla_{OXA-51}).

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INTRODUCTION

Acinetobacter baumannii, a gram-negative coccobacillus, was once thought to be an opportunistic pathogen but is now recognized as a significant cause of health care-associated infections. *A. baumannii* has developed resistance to the most effective antimicrobial agents in recent years, resulting in a high rate of morbidity and mortality, especially in intensive care units in many countries. During the wars in Lebanon, Afghanistan, and Iraq, *A. baumannii* became one of the most common pathogens, causing multiple outbreaks of MDR infections among combatants.[1, 2] *A. baumannii* is one of the most common nosocomial microorganisms, particularly in intensive care units (ICUs), where it causes pneumonia, septicemia, urinary tract infection, nosocomial meningitis, wound infection, and skin infection, among other illnesses.[3]

A. baumannii, a key emerging pathogen of nosocomial infections, may form biofilms on both biotic and abiotic surfaces, as it is well known. which it uses to promote survival on indwelling patients medical instruments, hospital surfaces, or in other adverse circumstances.[4] Furthermore, Badmasti *et al.,*[5] found that genetic variation and expression play a role in both antibiotic resistance and biofilm formation. Furthermore, they hypothesized that understanding the relations between these two phenomena could lead to a better understanding of the mechanisms of *A. baumannii* persistence in hospitals and colonization of hospital equipment like catheters and mechanical ventilators. Even when the dose administered is in the susceptible range, the ability to change a biofilm may affect antibiotic susceptibility and clinical failure.[6]

Virulence factor of *A. baumannii* raises the bacterium's survival rate in the hospital setting, raising the risk of nosocomial infections and outbreaks. Extended-spectrum β-lactamases are a rapidly emerging group of enzymes that can degrade a wide range of β-lactamas antibiotics, including third generation cephalosporins and aztreonam. Infections caused by ESBL-producing pathogens are becoming more common each year, owing to insufficient antimicrobial treatment.[7]

MATERIAL AND METHODS

Isolation and Identification

A total of 251 clinical *A. baumannii* isolates were obtained from hospitals in Najaf Province and visits from various clinical cases (wounds, burns, blood, sputum, and CSF) were collected from hospitals in Najaf Province and visitors. The

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isolates are collected during the study period from the initial September, 2020 till the end of February, 2021. Identified *A. baumannii* isolates by diagnostic tests including biochemical tests, API- 20 E and VITEK-2 Compact System.

Detection of Some Virulence Factors in *A. baumannii*

Genotyping detection of biofilm formation and β-Lactamas

The PCR method was used to identify specific virulence genes. include (*bap* gene and *blaOXA-51* gene).

Phenotyping Detection of Biofilm Formation (Quantitative Biofilm) and β-Lactamas.

In this study tissue culture plate (TCP) method described by Stepanovic *et al*.,[8] was used for biofilm detection in *A. baumannii* and β-Lactamase production by (iodine assay) according to (Sykes and Mathew, 2006.[9]

Extraction of Bacterial DNA

This procedure was used in connection with a genomic DNA purification kit provided by the manufacturer (Geneaid, UK). The DNA-containing suspension was kept at -20°C until it was used as a PCR template.

PCR Amplifications

The detection of virulence genes was done using PCR amplification. Table 1 shows the descriptions and sequences of the PCR primers used in this study.[1]

RESULTS

The samples are collected randomly from hospitalized patients and visitors in the Najaf province their percentages were as shown in the Figure 1. *A. baumannii* isolates isolated in high percentage in the wound and burn was 10 (41.6%), urine 7(29.1%), blood 4(16.6%), septum 2(8.3%) isolate and the later is CSF only 1(4.1%). All sites of specimens are shown in the Figure 2.

In this study all 24 of *A. baumannii* isolates were positive for biofilm formation, these results showed (62.5%) from all types of samples are strong, while about (25%) from all types of samples are moderate biofilm and (12.5%) is weakly biofilm. and all bacterial isolates (24 isolate 100%) were able to resistant beta-lactamase antibiotics according to phenotypic detection. While Conventional Polymerase Chain Reaction (PCR) with a specific primer was used for detection of the presence of bla_{OXA-51}- and *bap* gene results are shown that all isolates (24) of *A. baumannii* from total of (251) specimens possess (100%) bla_{OXA-51}- and *bap* gene. So, these results show that all isolates (100%) have the ability to produce β-lactamase and biofilm associated protein as shown in Table 2.

DISCUSSION

The results of biofilm production in this study were substantially identical to those of a local study, showing that 15.6% of the 154 clinical isolates examined were weak biofilm producers, while 32.5% and 45.4% had moderate and strong biofilm forming abilities, respectively. Yang *et al.*[10]

Molecular identification of *A. baumannii* by the detection bla_{OXA-51}- and *bap* gene of *A. baumannii* isolates with a PCR product size single amplicon 353 bp as shown in Figure 3,[11] was observed a bla_{OXA-51}-like gene was detected in all isolates of *A. baumannii.*

Also the current results were parallel with most of the quondam studies; a matter the proves the occurrence of bla_{OXA-51}- gene in all clinical isolates of *A.baumannii*.[12,13] The confirmation by bla_{OXA-51}- primers and observing ~ 353 bp band in the agarose gel it was in agreement with most preceding studies,[14] in Egypt.[15] In addition to *bap* gene, biofilm production capacity depends on additional genes such as pili assembly system (*csu*) and *omp*A.[16,17] This study near the result to other study[18] that showed of *bap* gene is determined 92%.

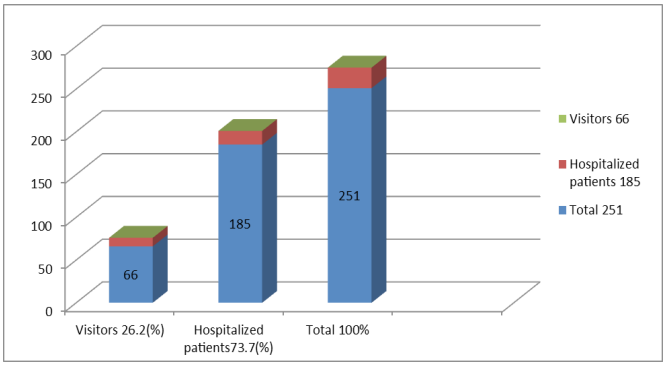

Figure 1: The percentages of clinical specimens according to visitors and hospitalized patients.

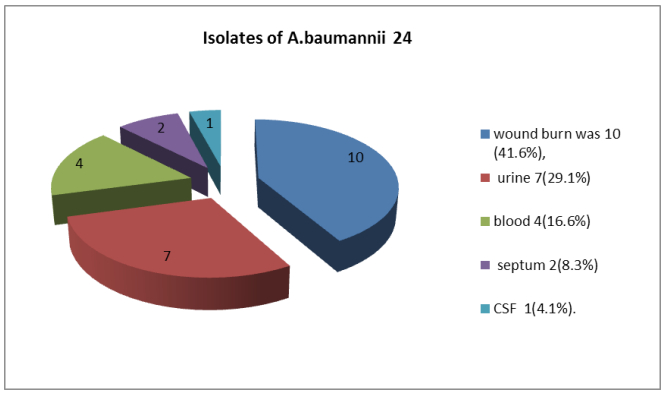

Figure 2: *A. baumannii* isolates according to the type of specimens

Table 1: The primers used in the current study for gene detection

Primer name	*Sequence*	*Annealing temperature (°C)*	*Product size bp*	*Reference*
bla_{OXA-51}- F	5`-TAATGCTTTGATCGGCCTTG-3`	52°C	353	(Joshi *et al*., 2017)
bla_{OXA-51}-R	5`-TGGATTGCACTTCATCTTGG-3`	58°C	353	
Bap-F	5'-ATGCCTGAGATACAAATTATTGCCAAGGATAATC-3'	58°C	560	
Bap-R	5'-AGGTGCTGAAGAATCATCATCATTAC-3'	58°C	560	

Table 2: Results for the virulence factor assessment of *A. baumannii* isolates

No.	*Biofilm production*	*β-lactamases production*	*bla_{OXA-51} gene*	*bap gene*
A.b1	+++	+	+	+
A.b2	++	+	+	+
A.b3	+	+	+	+
A.b4	+++	+	+	+
A.b5	++	+	+	+
A.b6	++	+	+	+
A.b7	++	+	+	+
A.b8	+++	+	+	+
A.b9	+++	+	+	+
A.b10	+	+	+	+
A.b11	+++	+	+	+
A.b12	++	+	+	+
A.b13	+++	+	+	+
A.b14	+++	+	+	+
A.b15	+++	+	+	+
A.b16	++	+	+	+
A.b17	+++	+	+	+
A.b18	+++	+	+	+
A.b19	+	+	+	+
A.b20	+++	+	+	+
A.b21	+++	+	+	+
A.b22	+++	+	+	+
A.b23	+++	+	+	+
A.b24	+++	+	+	+

*Biofilm production was interpreted as: weak (+), moderate (++) strong (+++).

Another study has[19] found from a total of 100 *A. baumannii* strains were isolated from hospitalized patients in Iran. 89% of them were positive for the *bap* gene (Figure 4). Our result disagrees by other research;[20] from 94 *A. baumannii* isolates showed *bap* (13.8%). Most bacterial isolates have the *Bap* gene, which codes for the biofilm associated protein. with biofilm-associated protein, a protein that spreads on the cell surface and plays a key function in adhesion to the host cell and non-living surfaces, as well as in biofilm development.[21]

CONCLUSIONS

According to the results of the present study, the following conclusions could be exposed

- *A. baumannii* posses (100%) *bla_{OXA-51} and bap gene.*
- the higher percentage of *A. baumannii* isolated from wounds & burn specimens in this study.
- All *A. bumannii* isolates had the ability to form biofilm and β-lactamases production

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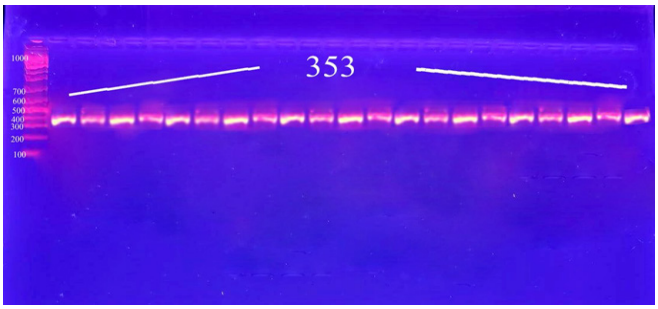

Figure 3: The amplification of *bla_{OXA-51}*- gene of *A. baumannii* samples were fractionated on 1.5% agarose gel(90 min at 100 volts) electrophoresis stained with Ethidim. Bromide M: 100 bp ladder marker. Lanes 1-20 resemble.

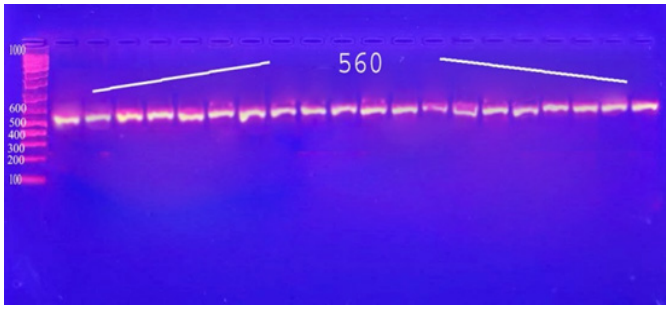

Figure 4: The amplification of *bap* gene gene of *A. baumannii* samples were fractionated on 1.5% agarose gel (90 minutes at 100 volts) electrophoresis stained with Eth.Br. M: 100 bp ladder marker. Lanes 1-20 resemble.

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