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 (25.0%) from all types of samples are moderate biofilm and (12.5%) is weakly biofilm. and all bacterial isolates (24 isolate 100%) were able to resist 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 blaOXA-51).

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

\textbf{Detection of Some Virulence Factors in \textit{A. baumannii}}

\textbf{Genotyping detection of biofilm formation and \(\beta\)-Lactamas}

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

\textbf{Phenotyping Detection of Biofilm Formation (Quantitative Biofilm) and \(\beta\)-Lactamas.}

In this study tissue culture plate (TCP) method described by Stepanovic et al.,\textsuperscript{8} was used for biofilm detection in \textit{A. baumannii} and \(\beta\)-Lactamase production by (iodine assay) according to (Sykes and Mathew, 2006).\textsuperscript{9}

\textbf{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\(^{\circ}\)C until it was used as a PCR template.

\textbf{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.\textsuperscript{1}

\section*{RESULTS}

The samples are collected randomly from hospitalized patients and visitors in the Najaf province their percentages were as shown in the Figure 1. \textit{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 \textit{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 \textit{bla}_{OXA-51}^-\textit{ and }\textit{bap} gene of \textit{A. baumannii} isolates with a PCR product size single amplicon 353 bp as shown in Figure 3,\textsuperscript{11} was observed a \textit{bla}_{OXA-51}^-\textit{like} gene was detected in all isolates of \textit{A. baumannii}.

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

\section*{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 \textit{et al.}\textsuperscript{10}

Molecular identification of \textit{A. baumannii} by the detection \textit{bla}_{OXA-51}^-\textit{ and }\textit{bap} gene of \textit{A. baumannii} isolates with a PCR product size single amplicon 353 bp as shown in Figure 3,\textsuperscript{11} was observed a \textit{bla}_{OXA-51}^-\textit{like} gene was detected in all isolates of \textit{A. baumannii}.

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
\textbf{Primer name} & \textbf{Sequence} & \textbf{Annealing temperature (°C)} & \textbf{Product size bp} & \textbf{Reference} \\
\hline
\textit{bla}_{OXA-51}^-\textit{ F} & 5'-TAATGCTTTGATCGGCCTTG-3' & 52°C & 353 & (Joshi \textit{et al.}, 2017) \\
\textit{bla}_{OXA-51}^-\textit{ R} & 5'-TGGATTGCACTTCATCTTGG-3' & 58°C & 353 & \\
\textit{Bap} F & 5'-ATGCCGTGAGATAAAAATTTGCCAAGGATAATC-3' & 58°C & 560 & \\
\textit{Bap} R & 5'-AGGTGCTGAAGAATCATCATTACG-3' & 58°C & 560 & \\
\hline
\end{tabular}
\caption{The primers used in the current study for gene detection}
\end{table}

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

Figure 2: \textit{A. baumannii} isolates according to the type of specimens.

1 Joshi \textit{et al.}, 2017
10 Yang \textit{et al.}, 2020
11 Joshi \textit{et al.}, 2017
12 Stepanovic \textit{et al.}, 2012
13 Joshi \textit{et al.}, 2017
14 Sykes and Mathew, 2006
15 Stepanovic \textit{et al.}, 2012
16 Stepanovic \textit{et al.}, 2012
17 Stepanovic \textit{et al.}, 2012
18 Stepanovic \textit{et al.}, 2012
Another study has 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; 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.  

CONCLUSIONS

According to the results of the present study, the following conclusions could be exposed
• A. baumannii posses (100%) blaOXA-51 and bap gene.
• the higher percentage of A. baumannii isolated from wounds & burn specimens in this study.
• All A. baumannii isolates had the ability to form biofilm and β-lactamases production

REFERENCES

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