Molecular Detection of Some Genes Responsible for Biofilm Formation in *Acinetobacter Baumannii* Isolated from Different Sites of Infection

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Abstract
baumannii is a G -ve bacteria, non-lactose fermenter, its increasingly recognized as a major pathogen causing infections. Methods: Fifty- six of A.baumannii was isolated from 100 samples of different sources (sputum, burns, bed sore , blood, wound and urine) in both sexes. These isolates were diagnosed by VITEK -2 system. Microtiter plate method used to detect biofilm formation. DNA extraction of 56 A. baumannii isolates, then molecular detection of some biofilm genes (bfmR and bfmS) were done by polymerase chain reaction. Results: The highest percentage of A. baumannii isolation (42%) was from sputum. These Isolates were divided into three groups according on biofilm production: strong, moderate, weak biofilm formation .Detection of biofilm formation genes revealed 38 (67.86%) positive bfmR gene versus 18 (32.14%) negative result. As well as 49(87.50%) Those isolates had the gene. bfms versus 7 (12.50 %) not carried the same gene.

Keywords: Acinetobacter baumannii, biofilm formation, bfmR, bfmS genes

1. Introduction

The gram-negative coccobacillus A. baumannii was once regarded as an opportunistic pathogen (1). which is playing a crucial role as the primary cause of infections in healthcare facilities. (2). Opportunistic pathogen A.baumannii, which is highly prevalent in immune-compromised humans, especially those who have had protracted hospital stays. Usually associated with aquatic environments(3). In current years, A. baumannii has created a high incidence rate of morbidity and death, particularly in the intensive care unit (ICU) in many countries, and has become resistant to the majority of the most effective antimicrobial antibiotics. A. baumannii, a significant emerging nosocomial infection pathogen, is recognized for its capability to form biofilms on both biotic and abiotic surfaces(4). These bacterial strains now exhibit a higher level of resistance to antibiotics and antimicrobial stresses as a result of biofilm formation. This means that one of the strong virulence factors is biofilm. (5).
Acinetobacter genome had the ability to merge foreign DNA. All six isolates share 475 genes, however the corresponding ambient species lack these genes. (6). BfmR is necessary for survival in vivo, and it promotes growth and survival in human ascites fluid and serum, which is at least partially mediated by providing resistance to complement-mediated bactericidal activity. (7). (8) indicated that the improved biofilm development should be attributed to the up-regulation of csu and bfmR-bfmS (two genes that work together to generate pilus-like bordered structures, or Csu operon).

2. Methods

1.Specimen collection

Fifty- six of A.baumannii was isolated from 100 samples 0f different sources of infections (sputum, burns, Bed sore, blood, wound swabs and urine).

2.Bacterial identification

Samples included streaked on Blood agar, MacConkey agar and Drigalski Lactose agar. VITEK-2 compact system (BioMerieux \DensiCHEK plus (France) used to confirm the diagnoses of isolates.

3.PCR method:

The conventional PCR with 25µl of Master mix reaction and optimized primer for each gene was mixed well for amplifing by (PCR). PCR Program was 95C for 2min. Initial Denaturation, 95C for 30sec Denaturation, 55C for 30secAnnealing, 72C for 1min Extension, 72C for 5min Final extension repeated 30 cycles. Then 1.5% agarose gel has been run horizontally in TBE buffer. Samples of DNA was mixed with loading at 150 v, for 30min. Bands are visualized by using an (UV) transilluminator. Table (1) shows a list of Primers used in this study.

doi.org/10.31838/hiv23.02.84 Received: 05.10.22, Revised: 02.11.22, Accepted: 04.12.22

Table (1): Shows a list of Primers used in this study					
Primer Name	Seq. 5`. 3`		Annealing Temp. (℃)	Product size (bp)	Reference
bfmR	F	GAAGTTGGTGTAGAAACCGATG	55	557	(9)
	R	GGATTTTCAGGATCATCGCC 557			
bfmS	F	CATTAGTGAAGGAGTCGCTCG	55	990	(9)
	R	GGTGTACCCTGCTCTAGTTTT			

4.Biofilm detection method: was adapted from O'Toole (2011) (10), overnight cultures of the bacteria in Brain Heart Infusion Broth (BHIB) ,the bacteria cultured after diluted and adjusted by McFarland tube 0.5, then 200 µl of each bacterial dilution deposited in three wells of a sterile 96-well polystyrene microliter plate and incubated under constant conditions at 37°C for 24 h. (11) .Biofilm production measured the optical density (O.D) by ELISA reader then calculate the results as follows: OD630 (bacteria) divided on 3 then Biofilm = OD630 (bacteria) – OD630 (control).Biofilm producing strains were scored as strong ,moderate, and weak as mentioned(12). The results was red as follow: Weak: ODc<OD≤2xODc, Moderate: 2xODc<OD≤4xODc, Strong: OD>4xODc(13).

3. Result and Discussion

The results of this study documented the 42 (42.0%) cases of A.baumannii bacterial isolates out of 100 were picked up from sputum samples, followed by 29 (29.0%) cases of isolates were isolated from burn samples, while the less cases were picked up from urine samples 3 (3.0%) from total study cases (100%), these differences statistically were highly significant (P≤0.0001) as illustrated in figure 1.

The results show the highest percentage of isolation (42%) was of sputum this result agreement with two Iraqi and international studies results shown highly respiratory tract infection by(14).

The similarities and differences in sites and distribution of infection of bacteria show difference from country to another due to patients condition number of patients examined ,health practices, personal hygiene environment of condition and laboratory procedures(15).

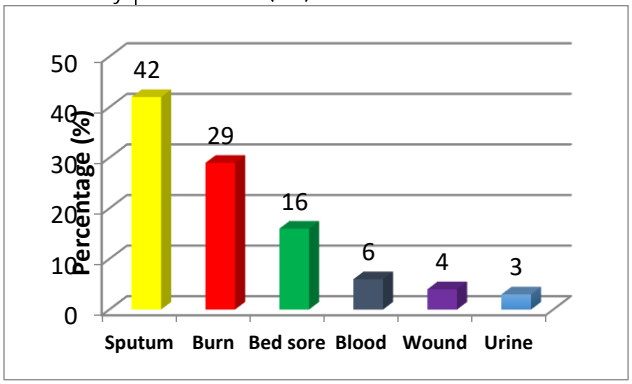

Figure 1: Percentage of A.baumannii isolated from different clinical samples

bfmR gene

The results of documented 38 (67.86%) of A.baumannii positive bfmR gene versus 18 (32.14%) negative result, these differences in the frequency and percentages of prevalence of genes were highly significant (P≤0.01) as arranged in table 2 and figure 2 bfmR suppression would have the combined benefit of greatly reducing in vivo survival and boosting the susceptibility of A. baumannii to other antibiotics, which is an essential and highly appealing feature of the proteins. (16).

Table 2: Results of distribution of bfmR gene of *A.baumannii*		
bfmR gene	No	Percentage (%)
Positive	38	67.86
Negative	18	32.14
Total	56	100%
Chi-Square (χ^2)	---	7.142 **
P-value	---	0.0075
** (P≤0.01).		

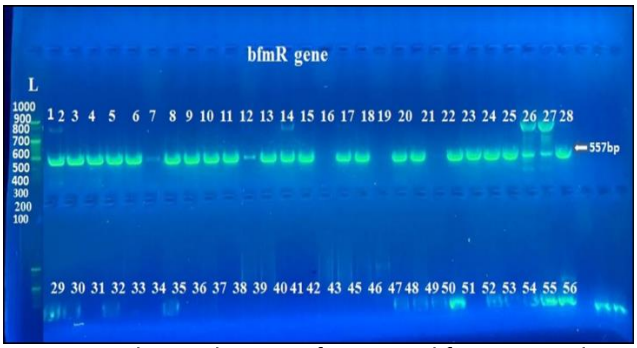

Figure (2) Electrophoresis of PCR amplification produce biofilm gene (bfmR) A. baumannii , expected amplification products are 557bp (lanes1-56:samples, lane L: Ladder100- 1000bp,1.5%agrose gel and 150 Volt for 20 min. in TBE buffers.

It is known that the A. baumannii bfmRS system regulates a number of phenotypes. Initially, bfmR was discovered because it could encourage the development of biofilms and control the production of the csu pili, which are important for adhering to inorganic surfaces. (17). The Csu operon, which is crucial for the attachment of planktonic cells to the abiotic surface, colony formation, and ultimately the entire development of biofilm, was expressed at low levels in the bfmR mutant, which resulted in a dramatic reduction in biofilm and pellicle formation.(18). Two components, bfmS and bfmR, control the Csu operon. (19).complementation of the bfmS gene deletion required for pili assembly and biofilm formation(20).Tomaras AP,Flagler MJ,DorseyJA, Actis LA. Characterization of the Acinetobacter baumannii two-component regulatory system (21).

Detection of *bfmS* gene

The results revealed there were 49(87.50%) of A.baumannii isolates carried the gene bfms versus 7 (12.50 %) not carried the same gene ,these differences in the frequency and percentages of prevalence of genes were highly significant (P≤0.01) as arranged in table (3) and figure (3).

Table (3): Results of distribution of bfmS gene of A.baumannii isolates		
bfms gene	No	Percentage (%)
Positive	49	87.50
Negative	7	12.50
Total	56	100%
Chi-Square (χ2)	---	..31.50 **
P-value	---	0.0001
** (P≤0.01).		

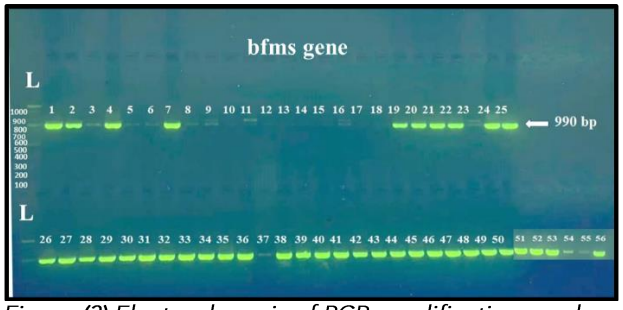

Figure (3) Electrophoresis of PCR amplification produce biofilm bfms gene A. baumannii, expected amplification products are 990bp (lanes1-56: samples, lane L: Ladder100- 1000bp,1.5%agrose gel and 150 Volt for 20 min). using TBE buffers.

BfmS genes, as demonstrated by (22) who showed that the bfmS gene's increased expression boosted the level of csu expression. (chaperon usher pili) locus and subsequently forming pili for twitching motility in A. baumannii. the increased expression of all genes after the addition of 100 mol/L C6-HSL to A. baumannii culture media, belonged to the csu locus together with the chaperone-usher regulators BfmS, indicating a strong connection between quorum sensing and the formation of type 1 pili.(23).

In A. baumannii, the bfmRS operon controls the K locus genes that produce capsular exopolysaccharide. (24).

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