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Distribution of Four Biofilm Associated Gene among *A. baumannii* by in Silico-PCR

J. D. Monesh Babu [a#], A. S. Smiline Girija [a*], P. Sankar Ganesh [a] and J. Vijayashree Priyadharsini [a]

[a] *Department of Microbiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Author JDMB carried out the literature search, data collection, data analysis and manuscript writing. Author ASSG conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. All the authors have equally contributed in developing the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: *A.baumannii* is an opportunistic pathogen known for its efficient biofilm formation that is attributed for its virulence. *Acinetobacter baumannii* is an inhabitant of oral biofilms as well. Many gene operons are involved in the biofilm formation that need to be monitored frequently.
Aim: The aim of the present study was to detect the distribution of four biofilm associated genes among *A.baumannii*.
Materials and Methods: Four biofilm forming genes viz., bfms, ptk, pgaB, and fimH of *A.baumannii* were selected. Forward and reverse primers of those four genes were used for in-silico PCR amplification. 19 strains of *A.baumannii* set as default on the server were chosen and the amplicon bands were observed
Results: The present investigation documents the distribution of four vital biofilm associated gene among 19 different strains of *A.baumannii* among which bfms was distributed at a higher frequency followed by pgaB and ptk
Conclusion: The finding of the study suggests the presence of pgaB, bfms, ptk among the 19 different strains of *A.baumannii*. However further experimental validation must be done to frequently monitor the presence of the genes among the clinical strains of *A.baumannii*.

[#] *Undergraduate Student;*
**Corresponding author: E-mail: smilinegirija.sdc@saveetha.com;*

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1. INTRODUCTION

Acinetobacter baumannii is a typically short, almost round, rod-shaped gram-negative bacterium. It is an opportunistic pathogen in humans, affecting people with compromised immune systems, and is becoming increasingly important as a nosocomial pathogen [1]. While other species of the genus *Acinetobacter* are often found in soil samples, it's almost adapted to survive in harsh hospital niches. *A. baumannii,* a Gram-negative, the most successful nosocomial pathogen within a short time period with 2–10% of death rate recorded among patients with chronic tract infections, bacteremia, pneumonia,, and critically ill-patients in ICU. The World Health Organization [WHO] has provided an alert about the multi-drug resistance in *A. baumannii* and many earlier studies have also reported the resistant patterns in the clinical strains [2-5].

The biofilms produced by the bacteria were found to be reservoirs of pathogens related to pneumonia and chronic obstructive pulmonary disease. Where, biofilms are a collective sites of one or more sorts of microorganisms that usually grow on on both biotic and abiotic surfaces, as well as on implanted medical devices like catheters and pacemakers [6]. Microorganisms that form biofilms include bacteria, fungi, and protists. Biofilm formation is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation, resulting in an alteration in the phenotype of the organisms with respect to growth rate and gene transcription [7]. The Bfm RS two-component system plays a vital role in pathogenesis and antimicrobial resistance of *A. baumannii* via the regulation of bacterial envelope structures. This study investigated the role of the sensor kinase, BfmS, in localization of outer membrane protein (OmpA) within the outer membrane, and the production of outer membrane vesicles (OMVs) [8,9].

Vital genes like *fimA* gene encodes for the massive secondary unit while, the *fimF* and *fimG* genes encode the tiny subunits. *fimH* gene encodes the highest of the cilia that are sensitive to the manus. *fimC* gene encode the attached protein and aid *fimD* to bind with outer membrane proteins. The fimH gene is a crucial virulence agent for bacteria that encodes the sort 1 fimbriae, that helps bacteria to bind to the surface of host cells that cause injury [10,11]. In addition, *A.baumannii* contains a pgaABCD locus that encodes proteins that blend cell-related poly-β-(1-6)- N-acetylglucosamine (PNAG). Different expansive consecutive investigations report that pgaABCD operon during *A.baumannii* and various other gram-positive and negative microscopic organisms also encode PNAG The polymerase chain reaction (PCR) is prime to biology and is the most vital practical molecular technique for the lab. The principle of this system has been further used and applied in many other simple or complex macromolecule amplification technologies [12,13]. In parallel to laboratory experiments for macromolecule amplification technologies, in silico or virtual (bioinformatics) approaches are developed, among which in silico PCR analysis which is a promising approach from the earlier studies [14]. The present investigation is thus undertaken to evaluate the frequency of four biofilm associated genes among the 19 different strains of *A. baumannii* by computational approach.

2. MATERIALS AND METHODS

2.1 Study Setting

This observational study was carried out using a computational approach, and the institutional approval was obtained (SRB/IHEC/SDC/UG-1895/21/163). The present study was done with the help of in-silico amplification tools (computerised in silico.ehu.es). By the use of pre-identified forward and reverse primers of the four genes (bfms, ptk, pgaB, fimH) [15] the length of the base pairs and the number of bands were determined in 19 strains of *A.baumannii* using the in silico amplifier web page. And with the help of these primers obtained we can compare and determine the virulence factors of each genome.

2.2 Evolutionary Analysis by Maximum Likelihood Method

The developmental history was derived by utilizing the Maximum Likelihood strategy and Tamura-Nei model [16]. The tree with the most elevated log probability (- 1116.82) has appeared. Introductory tree(s) for the heuristic inquiry were obtained consequently by applying Neighbor-Join and BioNJ calculations to a network of pairwise distances assessed utilizing the Tamura-Nei model, and afterward choosing the geography with prevalent log probability esteem. investigation included 14 nucleotide groupings. Codon positions included were

1st+2nd+3rd+Noncoding.There were a sum of 365 situations in the last dataset investigations were directed in MEGA X.

3. RESULTS

The investigation on the prevalence of the drug resistant genes from 19 different strains of *A.baumannii* using an in-silico amplification server was promising. The results showed the starting position of the amplification in the chromosome or plasmid and the length of each amplicon. Amplicons obtained in each chromosome or plasmid have been tabulated [Table 1] with target genes, primers used, sequenced of primer (5' to 3'), annealing temperature, estimated size of base pair and the frequency of the target genes among the study strains. In the present study, BfmS showed an amplicon size of 1428 bp. PtK showed an amplicon size of 597 bp [Table 1]. PgaB showed an amplicon size of 490bp. fimH showed no bands (Figs. 1-3). Nearly 52.63% of distribution for bfms, 21.05% of distribution for pgaB and about 10.52% of distribution for ptk was observed among the selected 19 different strains of *A.baumannii*. Further assessement of the evolutionary pattern of the distributed genes among the strains was done as given in Fig. 4.

Table 1. Showing the target genes, PCR conditions of the biofilm genes selected for the study

Target Genes	Primers sequences (5–3)	Annealing Temperature (°c)	DNA amplicon Size (bp)	Bands
bfmS	TTGCTCGAACTTCCAATTTATTATAC TTATGCAGGTGCTTTTTTATTGGTC	60	1428	10
ptk	GGCTGAGCATCCTGCAATGCGT ACTTCTGGAGAAGGGCCTGCAA	57	597	2
pgaB	AAGAAAATGCCTGTGCCGACCA GCGAGACCTGCAAAGGGCTGAT	57	490	4
fimH	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	60	870	No bands

Table 2. Showing the distribution of the bfmS among the selected strains of *A. baumannii*

Strain name	Sequence id	Start position	End position	Base pair length
Acinetobacter baumannii 1656-2 chromosome	NC_017162	823749	825176	1428
Acinetobacter baumannii ACICU	NC_010611	793047	794474	1428
Acinetobacter baumannii ATCC 17978	NC_009085	889190	890617	1428
Acinetobacter baumannii BJAB07104	NC_021726	834877	836304	1428
Acinetobacter baumannii BJAB0868	NC_021729	830598	832025	1428
Acinetobacter baumannii D1279779	NC_020547	789219	790646	1428
Acinetobacter baumannii MDR-TJ	NC_017847	3143572	3144999	1428
Acinetobacter baumannii MDR-ZJ06	NC_017171	821690	823117	1428
Acinetobacter baumannii TCDC-AB0715	NC_017387	826988	828415	1428
Acinetobacter baumannii TYTH-1	NC_018706	1043958	1045385	1428

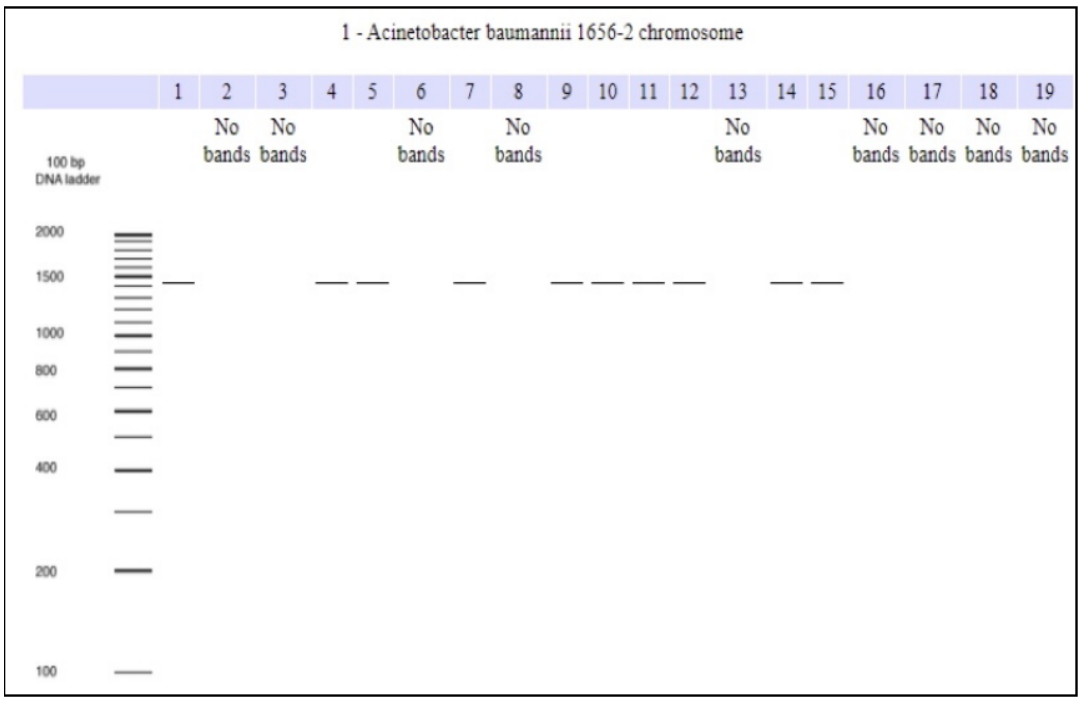

Fig. 1. Showing BfmS with an amplicon size of 1428 bp among the selected strains of *A. baumannii*

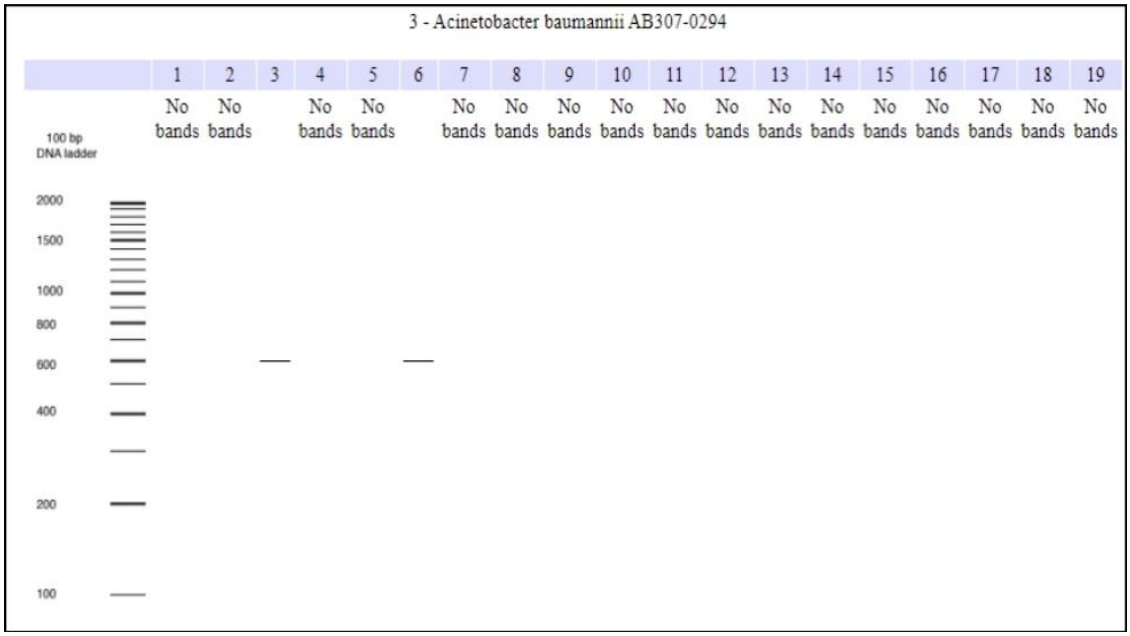

Fig. 2. Showing PtK with an amplicon size of 597bp among the selected strains of *A.baumannii*

4. DISCUSSION

A. baumannii and its associated virulence of biofilm formation attributes for the initiation and progression of the disease in the hospital environment. Many genetic determinants of biofilms are documented in the pathogenesis of *A.baumannii*. The present study is thus undertaken to determine the frequency of four vital biofilm forming genes among 19 different strains of *A. baumannii* using an in-silico amplification server. Polymerase chain reaction is used for primer designing and is used for selective amplification of the target genetic

determinants. Recent improvements in technologies have made it easy to regulate a high specific theoretical possibility of a successful PCR by modifying a high specific and sensitive primer instead of laboratory assay which is expensive [17]. There are several free web servers available which we can use to identify the possible outcomes of a target gene using their preexisting forward and reverse primers. Using the specific primers for each different gene, frequency of the distribution of the genes among the selected strains were evaluated.

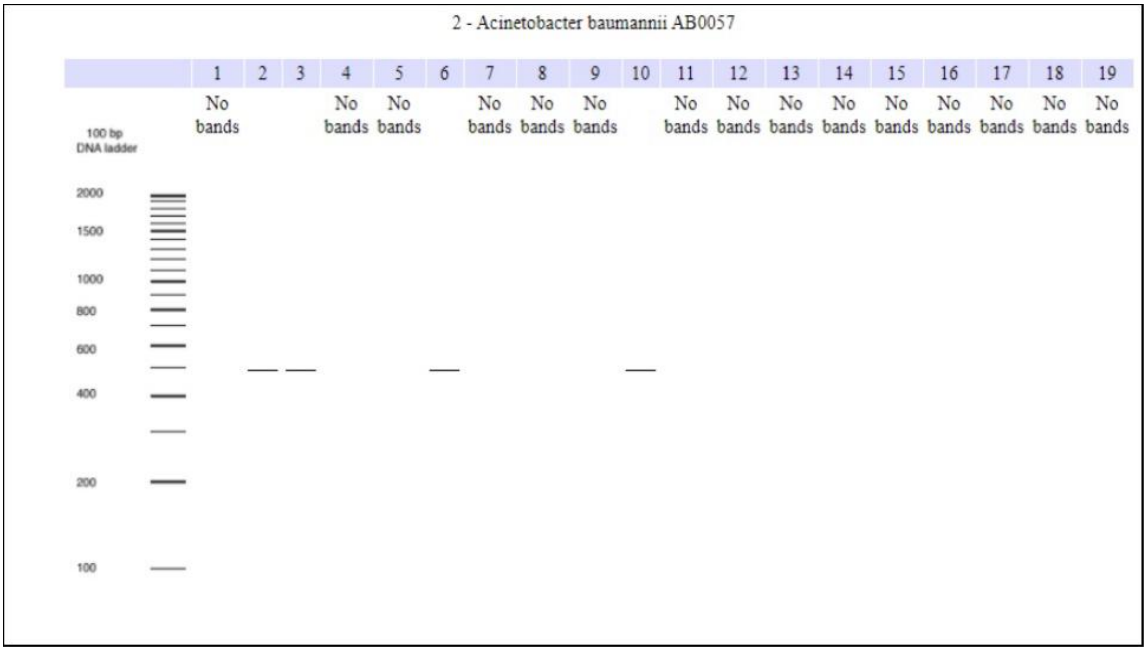

Fig. 3. Showing PgaB with an amplicon size of 490bp among the selected strains of *A. baumannii*

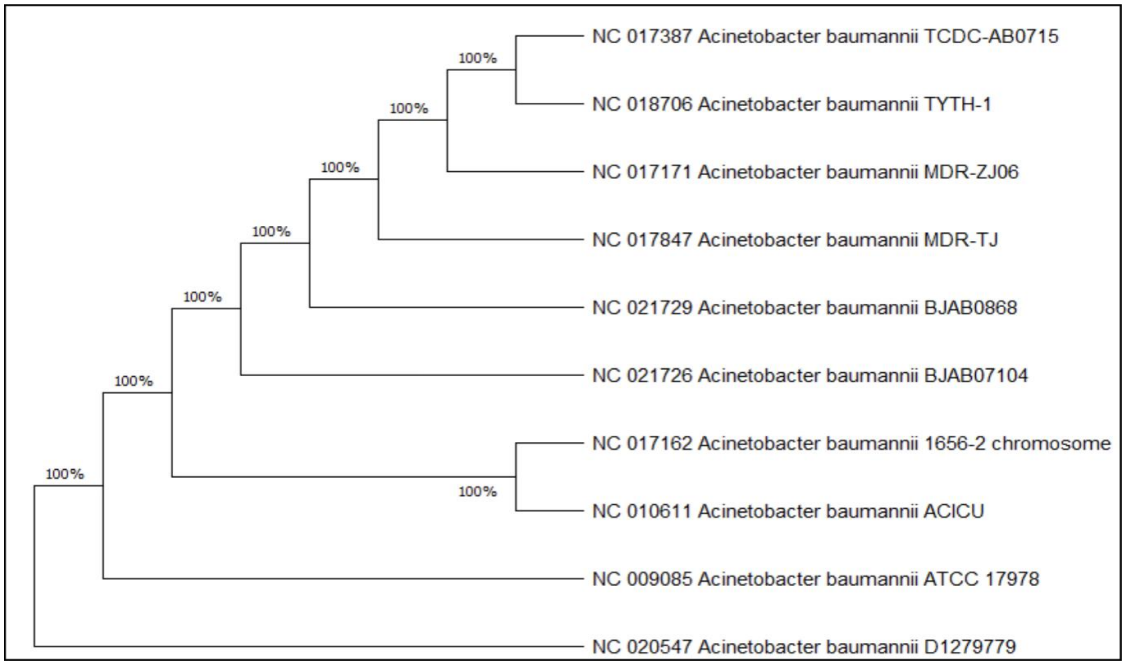

Fig. 4. Showing the phylogenetic evolutionary pattern of BfmS among the positive strains of *A. baumannii* using maximum likelihood strategy and tamura-nei model

From the results obtained, the present investigation documents the distribution Bfms at a higher frequency followed by pgaB and ptk and is correlating with earlier studies [18]. Such preliminary predictions also holds good to evaluated natural biocompounds against *A. baumannii* [19,20]. However fimH which was not reported in the study was highly responsible for virulence in earlier reports [11]. In the previous study on fimH gene, states that fimH can directly stimulate host cell signaling cascades that lead to bacterial internalization and exhibit greater frequencies when compared to this study. Type 1 fimbriae has also been described as a major factor in the formation of biofilm on the abiotic surface. More specifically, fimH was shown to be necessary to adhere to ephemeral surfaces in stable growth conditions. Thus fimH was used for detection among the *A. baumannii* strains.

Earlier studies on bfms in *A. baumannii* showed decreased biofilm formation, adherence to eukaryotic cells, and greater sensitivity to serum killing as compared to parent strains. The present study showed highest frequency of bmfs which correlates with many earlier reports [21]. In previous studies it has been revealed that the high frequency of biofilm forming genes among the XDR *A. baumannii* from ICU patients [22]. In-silico based computational approaches on the amplification of the target genes seem to be highly promising in the detection at a preliminary level for systemic diseases [23], viral diseases [24,25] and can also be applied for dental studies [26,27]. The limitation of the study was that the strains were selected from the default set-up in the tool and not with the clinical strains. Future prospects are set to experimentally validate the distribution of the genes among the clinical strains of *A. baumannii* and using the conventions PCR for amplification.

5. CONCLUSION

Finding of this study suggests the presence of 3 genes viz., *pgaB, bfms, ptk* with no distribution of *fimH* gene. However further experimental validation must be done to frequently monitor the presence of the genes among the clinical strains of *A.baumannii* to curtail the infections in healthcare settings.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval was obtained (SRB/IHEC/SDC/UG-1895/21/163) and has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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