DISTRIBUTION OF FOUR BIOFILM ASSOCIATED GENE AMONG *A.BAUMANNII* BY IN SILICO-PCR

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 and the present study is designed to assess the frequency of four vital biofilm forming genes among 19 different strains of *A.baumannii.*

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, fimH of *A.baumannii* were selected. Forward and reverse primers of those four genes as reported from earlier studies 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*.

Keywords: Biofilm; *A.baumannii;* novel pgaB ; bfms; ptk; innovative in-silico: environmental strains

Running title: Detection of biofilm associated genes among *A.baumannii*

INTRODUCTION:

Acinetobacter baumannii is a typically short, almost round, rod-shaped gram-negative bacterium. An opportunistic pathogen in humans, affecting people with compromised immune systems, and is becoming increasingly important as a nosocomial pathogen. While other species of the genus *Acinetobacter* are often found in soil samples, it's almost adapted to survive in harsh hospital niches. Although occasionally it's been found in environmental soil and water samples. *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 carbapenem-resistant *A. baumannii* which secures its place under the critical category (1, 2), (3).

The biofilms produced by the bacteria also are potential reservoirs of pathogens related to pneumonia and chronic obstructive pulmonary disease. Biofilms are collectively one or more sorts of microorganisms that will grow on many various surfaces (4). Microorganisms that form biofilms include bacteria, fungi, and protists. Biofilms are found growing on both biotic and abiotic surfaces and on implanted medical devices like catheters and pacemakers. 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 (5). The BfmRS 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) (6) (7).

Several studies have indicated that the few other vital genes like fimA gene encoding for the massive secondary unit while the fimF and fimG genes encode the tiny subunits, and therefore, the fimH gene encodes the highest of the cilia that are sensitive to the manus and therefore the fimC gene encode the attached protein that helps fimD encodes the outer membrane proteins and fimI encodes 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 (8). In addition, *A.baumannii* contains a pgaABCD locus that encodes proteins that blend cell-related poly-β-(1-6)-N-acetylglucosamine (PNAG) (9). 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 (10). In parallel to laboratory experiments for macromolecule amplification technologies in silico or virtual (bioinformatics) approaches are developed, among which in silico PCR analysis is promising. Our team has extensive knowledge and research experience that has translated into high quality publications (11, 12) (13) (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.

MATERIALS AND METHODS:

Study setting: This study was carried out as an observational study using a computational approach. Institutional approval for carrying out the study was obtained (SRB NUMBER:IHEC/SDC/UG-1895/21/163). The present study was done with the help of in-silico amplification tools (computerised in silico.ehu.es). Use of pre-identified forward and reverse primers of the four genes (bfms, ptk, pgaB, fimH) we were able to determine their length of the base pairs and the number of bands observed among 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.

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 (16). 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 (17,18).

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 (Figure 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 in the present study among the selected 19 different strains of *A.baumannii*. We further assessed the evolutionary pattern of the distributed genes among the strains as given in figure 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

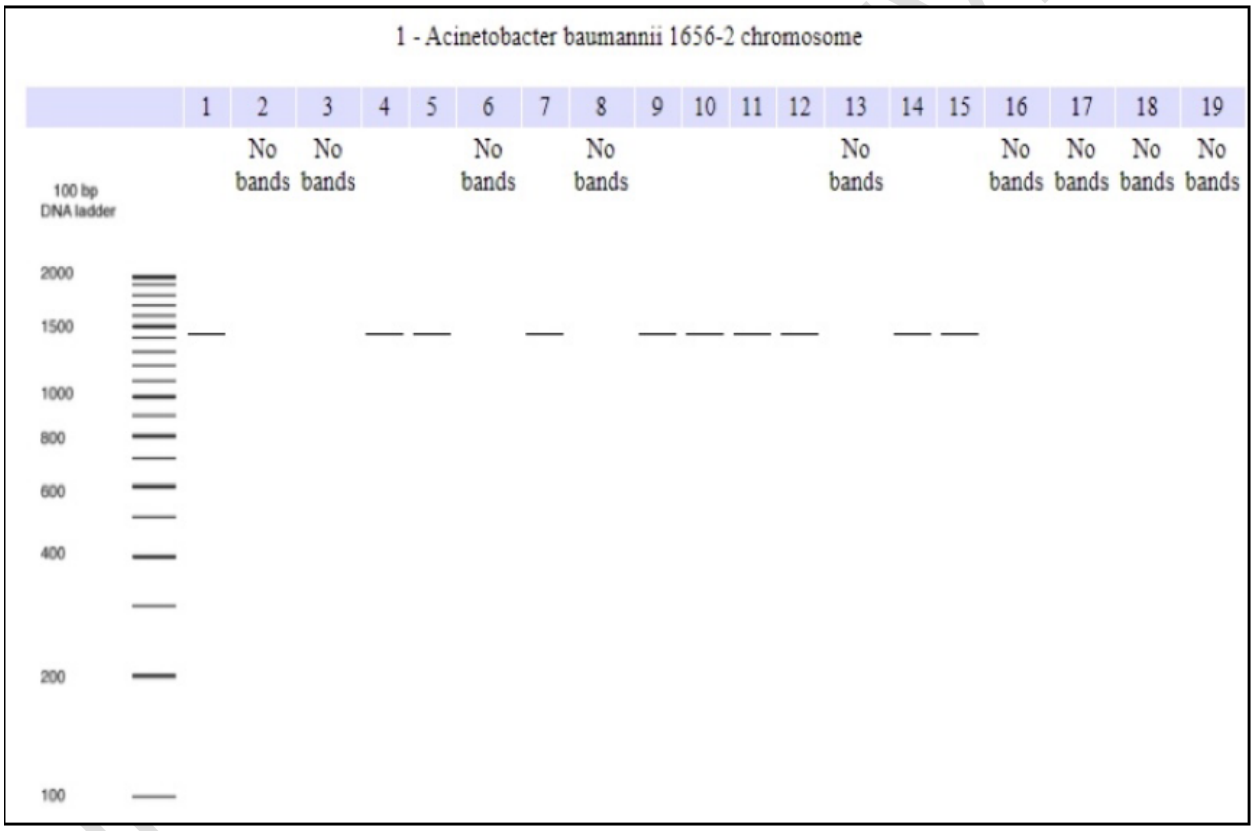

Figure 1 showing BfmS with an amplicon size of 1428 bp among the selected strains of *A.baumannii*

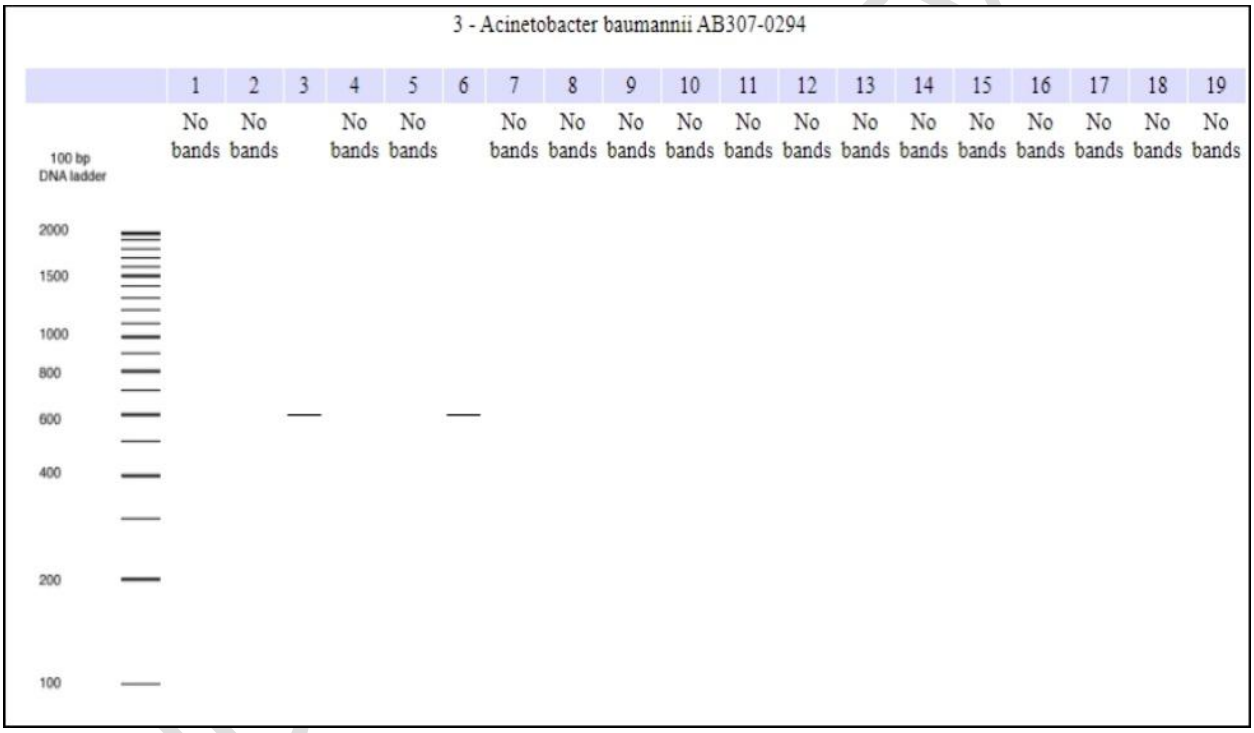

Figure 2 showing PtK with an amplicon size of 597bp among the selected strains of *A.baumannii*

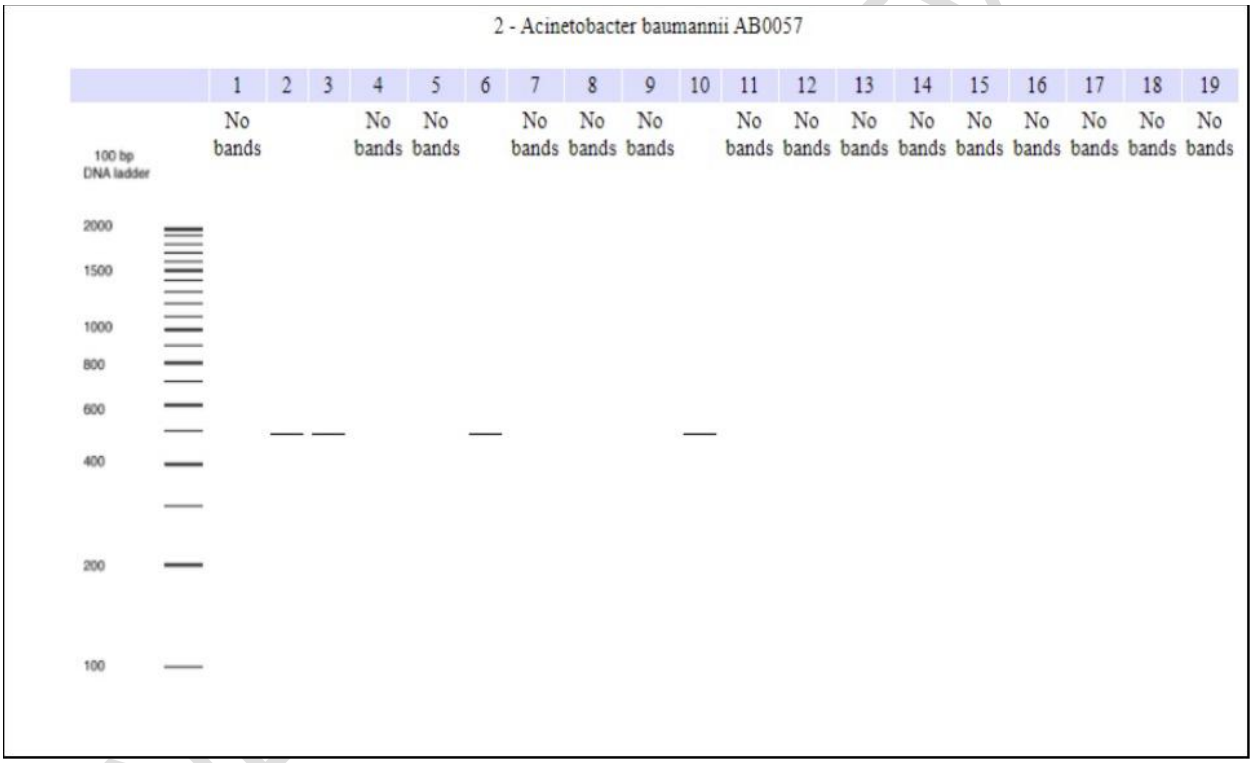

Figure 3 showing PgaB with an amplicon size of 490bp among the selected strains of *A.baumannii*

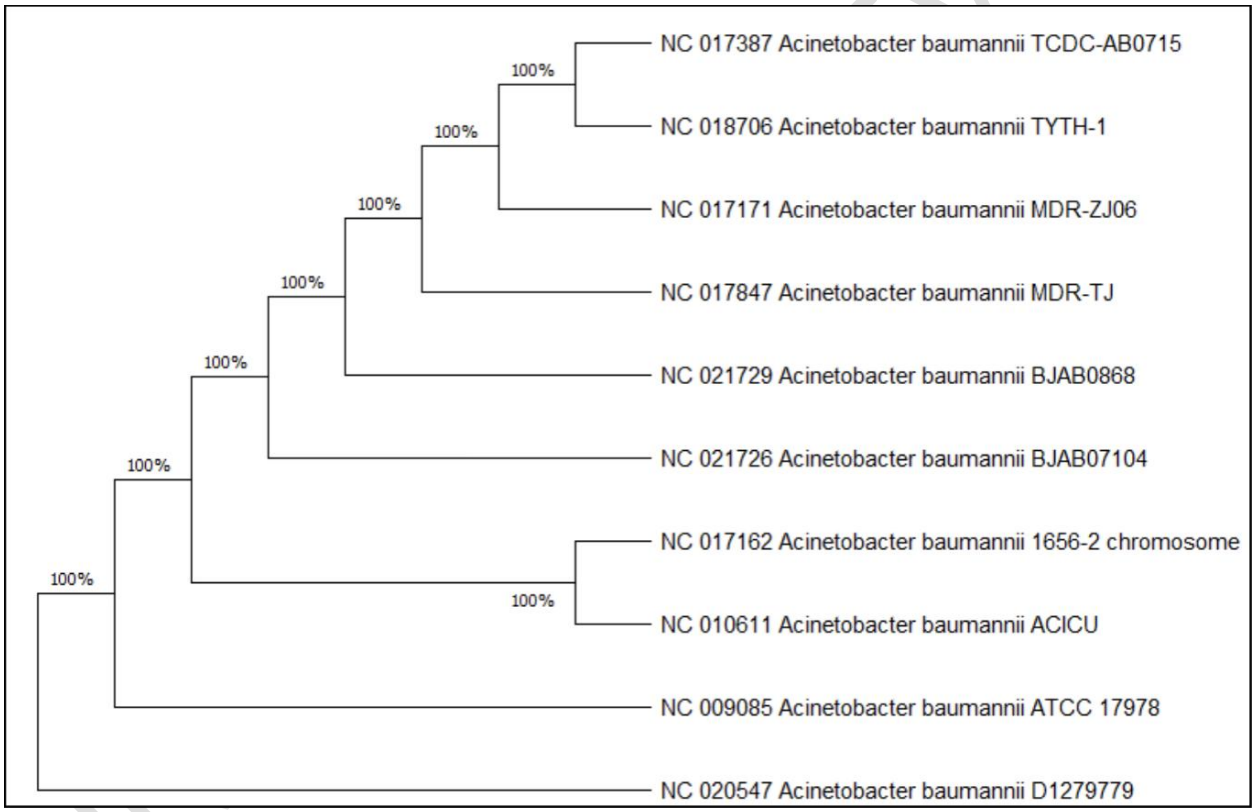

Figure 4 showing the phylogenetic evolutionary pattern of BfmS among the positive strains of *A.baumannii*

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 (19) (20). 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 as reported from earlier studies for each different gene, we evaluated frequency of the distribution of the genes among the selected strains (21).

From the results obtained, the present investigation documents the distribution Bfms at a higher frequency followed by pgaB and ptk these findings correlates with many earlier studies. However fimH which was not reported in the study was highly responsible for virulence in earlier reports. (22). 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 our present study (23). 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 (24) (25). Thus in the present study, we have chosen fimH for detection among the *A.baumannii* strains (26).

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. Our study had documented the highest frequency of bmfs which correlates with many earlier reports. (27–29).In previous studies it has been revealed that the high frequency of biofilm forming genes among the XDR *A. baumannii* from ICU patients. In the same line, presence of biofilm forming genes like pgAb and ptk has shown greater frequencies which is more significant in our study. In-silico based computational approaches on the amplification of the target genes seem to be highly promising in the detection at a preliminary level. The results were, however, correlated with earlier studies with few contrasting reports as well. 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 (30)(31)(32)

CONCLUSION:

The present study was undertaken to detect the frequency of the four important biofilm genes among 19 virulent strains of *A.baumannii*. The finding of the 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* and to curtail the infections in healthcare settings.

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