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

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Authors’ contributions
This work was carried out in collaboration among all authors. Author JDDB 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., bфмs, 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 bфмs was distributed at a higher frequency followed by pgaB and ptk.

Conclusion: The finding of the study suggests the presence of pgaB, bфмs, 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.

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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 membranes, 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 mutant. 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 pgaABC locus that encodes proteins that blend cell-related poly-β-(1-6)- N-acetylg glucosamine (PNAG). Different expansive consecutive investigations report that pgaABC 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 study 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 assessment 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**     | AAGAAAATGCCTGTCGGCAGCCA GCGAGACCTGCAAAGGGGCTGAT | 57                        | 490                    | 4     |
| **fimH**     | TGCAGAACGGATAGCGGTGGG 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             |
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 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 bfms 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/SRC/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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