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Detection of Drug Resistant Genes among *A. baumannii* by *In silico* PCR Method

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Authors' contributions

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

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ABSTRACT

Background: *Acinetobacter baumannii* is a gram-negative bacterium classified as an opportunistic pathogen in humans by the World Health Organization. Different genetic determinants contribute to multidrug resistance, and transform it as a nosocomial pathogen.
Aim: Using in-silico PCR, this analysis aims to characterize the 13 distinct drug resistant genes found in 19 virulent *A.baumannii* strains.
Materials & Methods: There were 11 *A.baumannii* multidrug resistance genes chosen. In-silico PCR amplification was performed using forward and reverse primers from the 11 genes described in previous research. The amplicon bands were detected in 19 strains of *A.baumannii* that were set as default on the server.
Results: Among the 13 multidrug resistance genes studied, *tet A, tet B, Sul 1, Sul 2, DfrA1, ISAba-1* and *ISAba-125* were detected among the 19 virulent strains of *A.baumannii.*

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Conclusion: The findings of the study documents the frequency of *tet A, tet B, Sul 1, Sul 2, DfrA1, ISAba-1* and *ISAba-125* like from the selected strains of *A. baumannii.* However, more experimental validation is needed in order to conduct routine surveillance on drug-resistant *A. baumannii* strains in hospital settings.

Keywords: A. baumannii; tetA; novel ISAba-1; resistance; innovative in-silico; environmental strains.

1. INTRODUCTION

Acinetobacter baumannii has become a particularly alarming pathogen in hospital environments and in various health-care settings [1]. *A. baumannii* is an oxidase-negative, non fermentative bacilli, gram-negative, non-motile and also in need of determination of its natural reservoir. It is however present in certain health settings and is a particularly effective coloniser of humans in the hospital settings. It has good nosocomial pathology due to the combination of its ecological intensity and the variety of resistance determinants [2]. *A. baumannii* has no demanding growth requirements and is able to grow at varying temperatures and pH conditions [3]. A number of carbon and energy sources are used by the diversified organism. These properties clarify Acinetobacter's tendency to survive in wet or dry hospital environments, leading therefore to transmission [4]. *A. baumannii* is multidrug-resistant [MDR] and can epidemically distribute strokes circulating in hospitals. A case of MDR *A. baumannii* has been documented with the wound infections also [5].

Among the various drug resistant genes, the OXA-β-lactamases have been established by the earliest β-lactamases. However, the D-β-lactamases were initially very uncommon and mostly mediated by plasmids. Since the 1980s *A. baumannii* have been present, which showed resistance to carbapenems together with OXA enzymes due to their similarity to plasmid-encrypted β-lactamases [6]. Also, almost all of the strains of *A. baumannii* had chromosomally encoded β-lactamase genes [7]. Furthermore, the *A. baumannii* developed by CHDL showed resistance to clavulanate and tazobactam and is evidenced by earlier reports [8]. *A. baumannii* resistant strains pose a serious threat to the medical practitioners [9]. In this line, *A. baumannii* showed resistance against tetracyclines including minocycline and doxycycline [10]. These drug resistant strains are often associated with the systemic infections with 71.9% of respiratory infections and 87.5% of bloodstream infections [11]. The efflux system found in these strains is known as a powerful mechanism for drug resistance, which reduces antibiotic accumulation leading to further resistance [12].

Additionally, hetero-resistant strains of ticarcillin-clavulanic acid, cefepime and cefpirome, are found among *A.baumannii* isolates [13]. The ability to up-regulate the expression of the ampC gene in combination with the multiple insertion elements has been an essential factor in *A. baumannii* resistance to cephalosporins [14]. In *A. baumannii* the molecular source of susceptibility to fluoroquinolone is chromosomally mediated and is associated with the defect in the gyrA gene [15]. Insertion genes, ISAba1also plays a crucial role in the transition and expression of resistance genes to carbapenem in *A. baumannii [16]*. This ISAba1 insertion factor has been reported to be associated with carbapenem-resistance genes blaOXA-51-like, blaOXA-23-like, and blaOXA-58-like [17].

Effective polymerase chain reaction [PCR] is the product of efficient primer design and selective amplification of the target gene loci. Advancing computational algorithms has helped us to measure the theoretical probability of a good PCR by developing extremely precise and responsive primers before beginning costly laboratory tests. Our team has extensive knowledge and research experience that has translate into high quality publications [18–22,23–27].

Thus this study is aimed to detect the 13 different drug resistant genes among the 19 strains of *A. baumannii* set as default in the *in-silico* PCR amplification server.

2. MATERIALS AND METHODS

Study Setting: This study was carried out in the Department of Microbiology, Saveetha Dental College and Hospital, Chennai as an observational study using a computational approach.

This is an original research study where we have selected 19 strains of *A.baumannii* set as default

in the *in-silico* PCR server. The genes of target were *qnrA, qnrB, qnrS, aac[6]Ib, tet A, tet B, Sul 1, Sul 2, Dfr A1, Dfr A5, ISAba-1, ISAba-4* and *ISAba-125*. Upon the amplification command, the server produced the amplicon bands for evaluation of the band size. From the amplicon bands, the frequency of the distribution of the drug resistant genes among the vital virulent strains of *A. baumannii* were evaluated and compared. Further evolutionary relationships were compared with the phylogenetic analysis as done in earlier reports [28,29].

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 *in-silico* based PCR amplification tool is an easier method to detect the presence of the target genes in the desired strains. It requires less manpower and easier to perform. 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 documented with target genes, primers used, sequence of primer [5' to 3'], annealing temperature, estimated size of base pair and the frequency of the target genes among the study strains. Among the 13 multidrug resistant genes we observed 63.15% positivity of *ISAba-1* 52.63% for *Sul1*, 42.10% for *Sul2*, 42.10% for *TetB*, 15.78% for *ISAba125*, 10.52% for *TetA* and 5.26% for *DfrA1* (Figs. 1-7). We further assessed the evolutionary pattern of the distributed genes among the strains [Figs. 8-11].

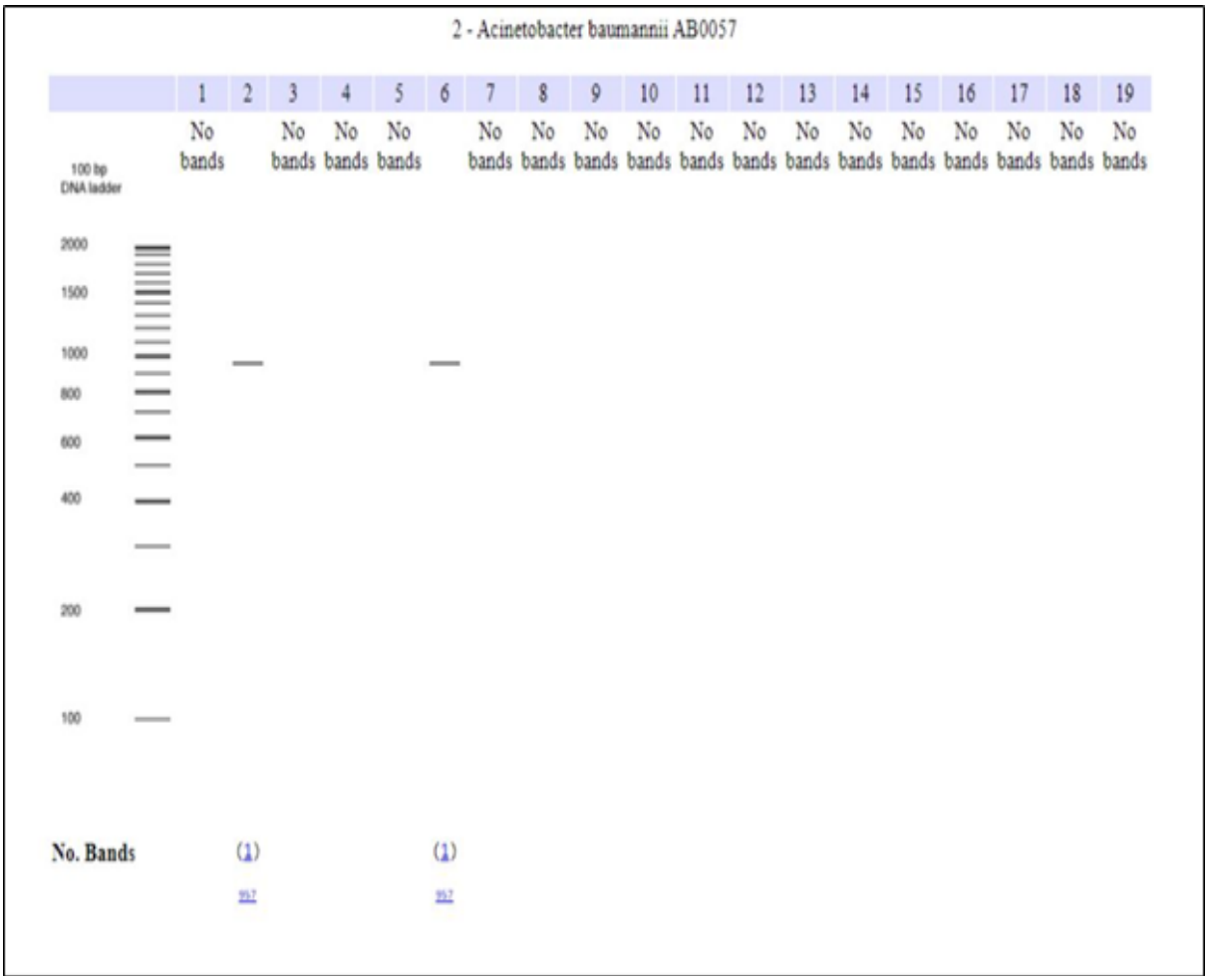

Fig. 1. Showing the positive band formation for *Tet A li*ke using in-silico PCR amplification among the strains of *A. baumannii*

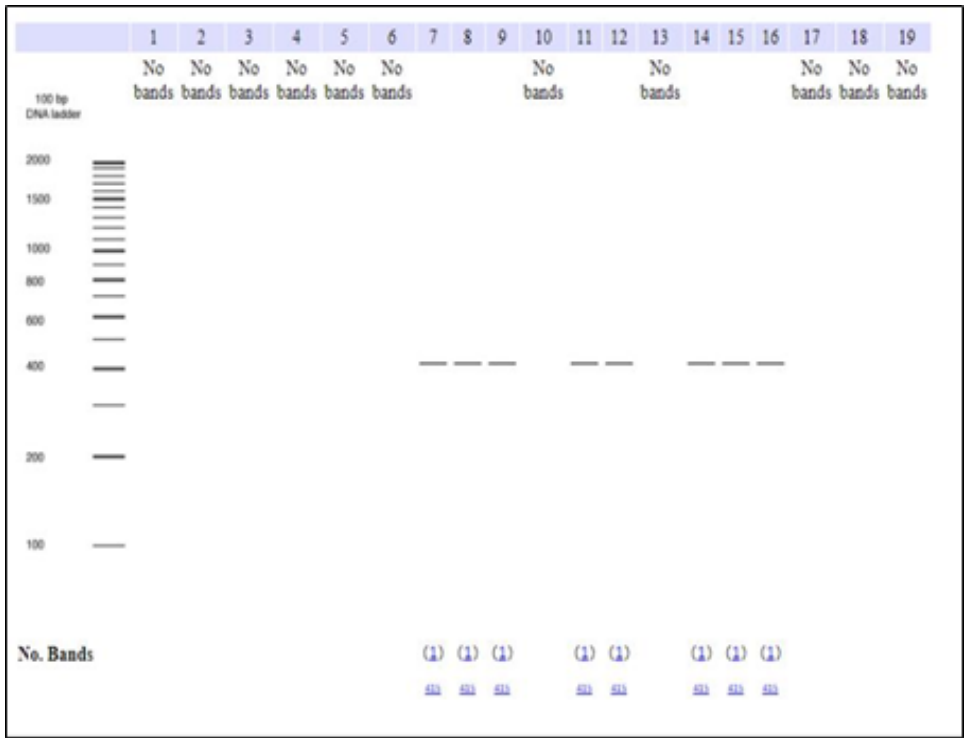

Fig. 2. Showing the positive band formation for *Tet B li*ke using in-silico PCR amplification among the strains of *A. baumannii*

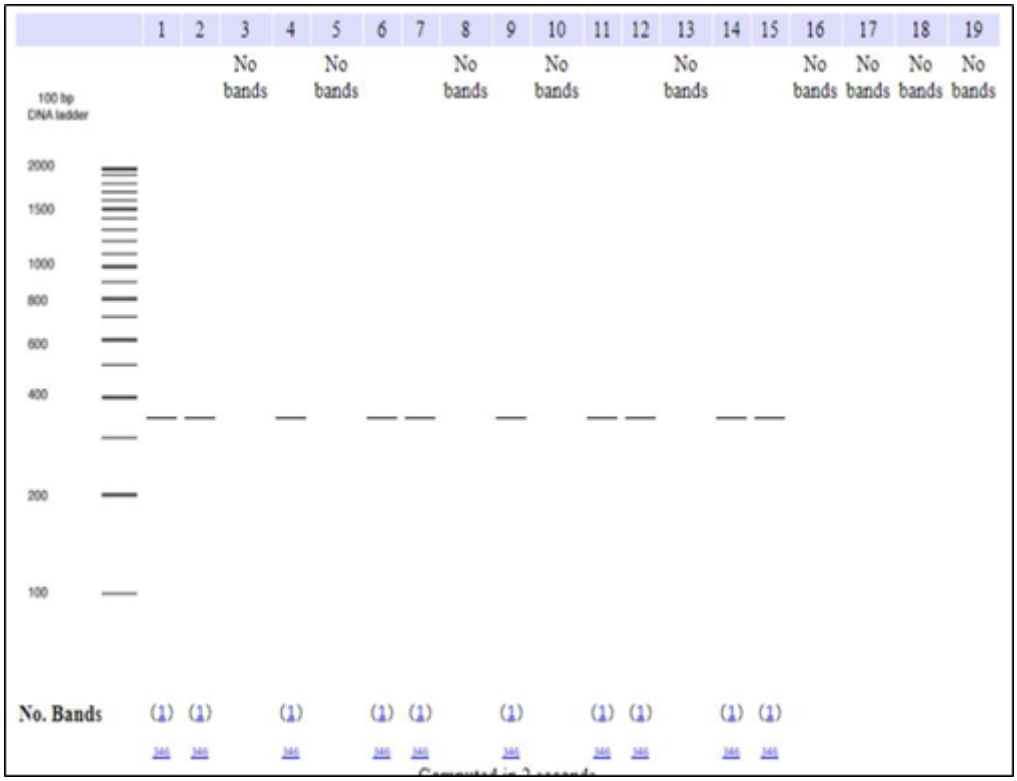

Fig. 3. Showing the positive band formation for *sul 1 li*ke using in-silico PCR amplification among the strains of *A. baumannii*

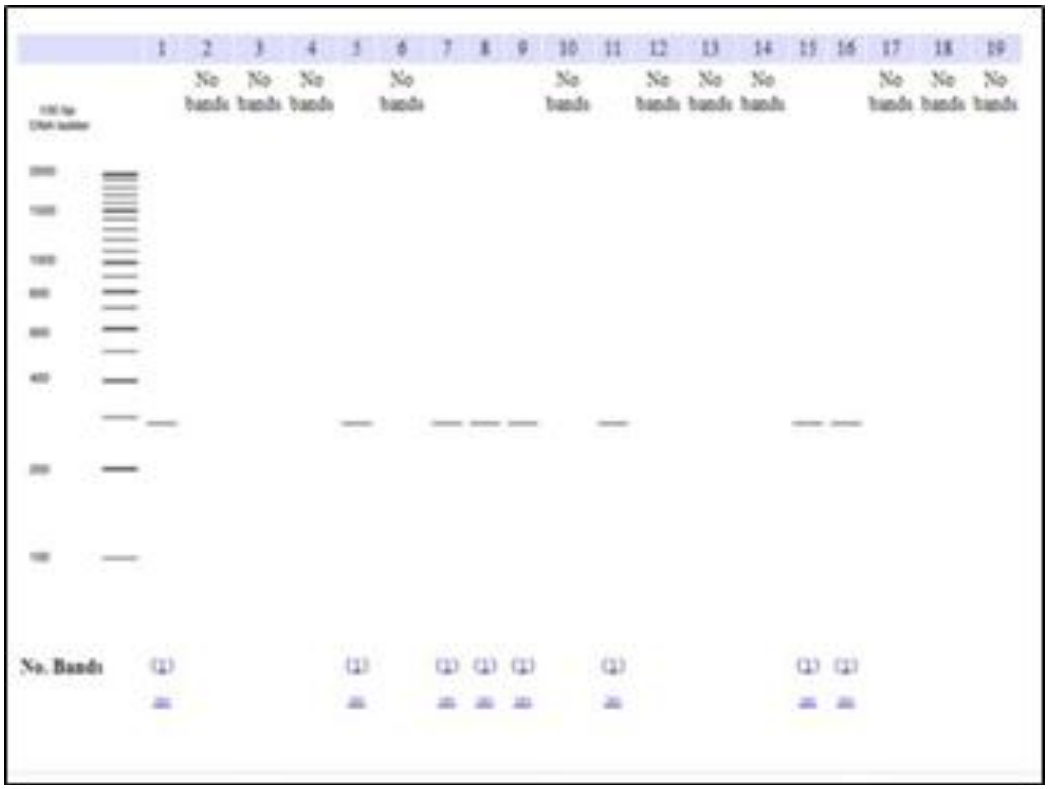

Fig. 4. Showing the positive band formation for *Sul 2 li*ke using in-silico PCR amplification among the strains of *A. baumannii*

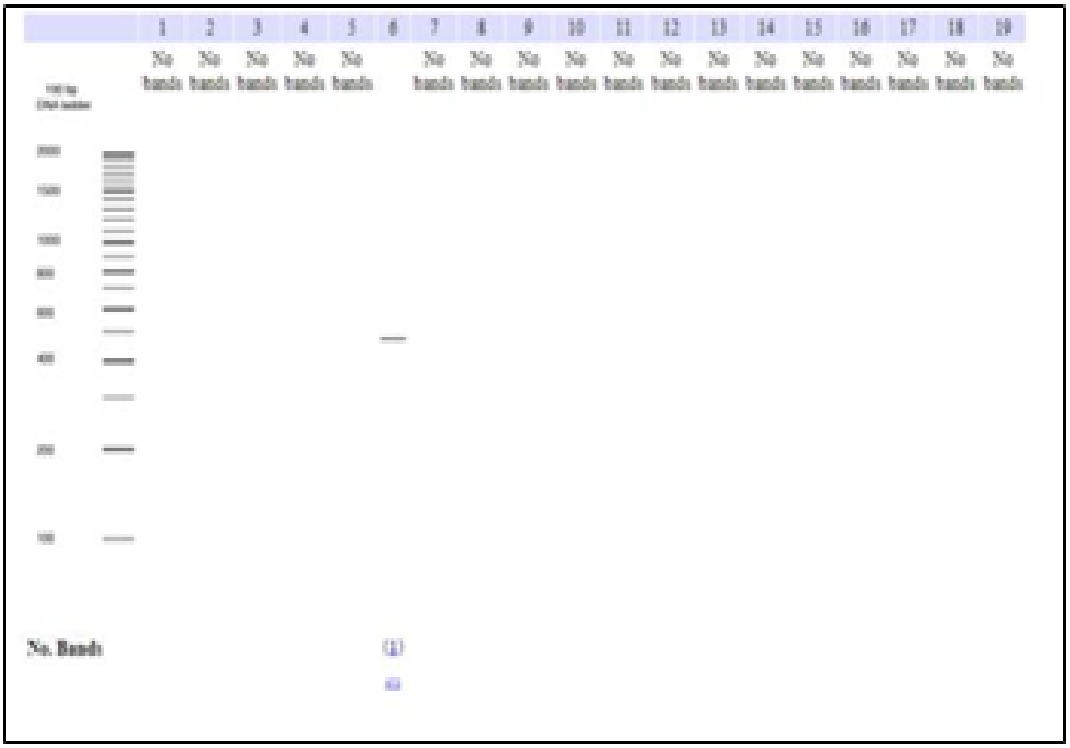

Fig. 5. Showing the positive band formation for *dfr A1 li*ke using in-silico PCR amplification among the strains of *A. baumannii*

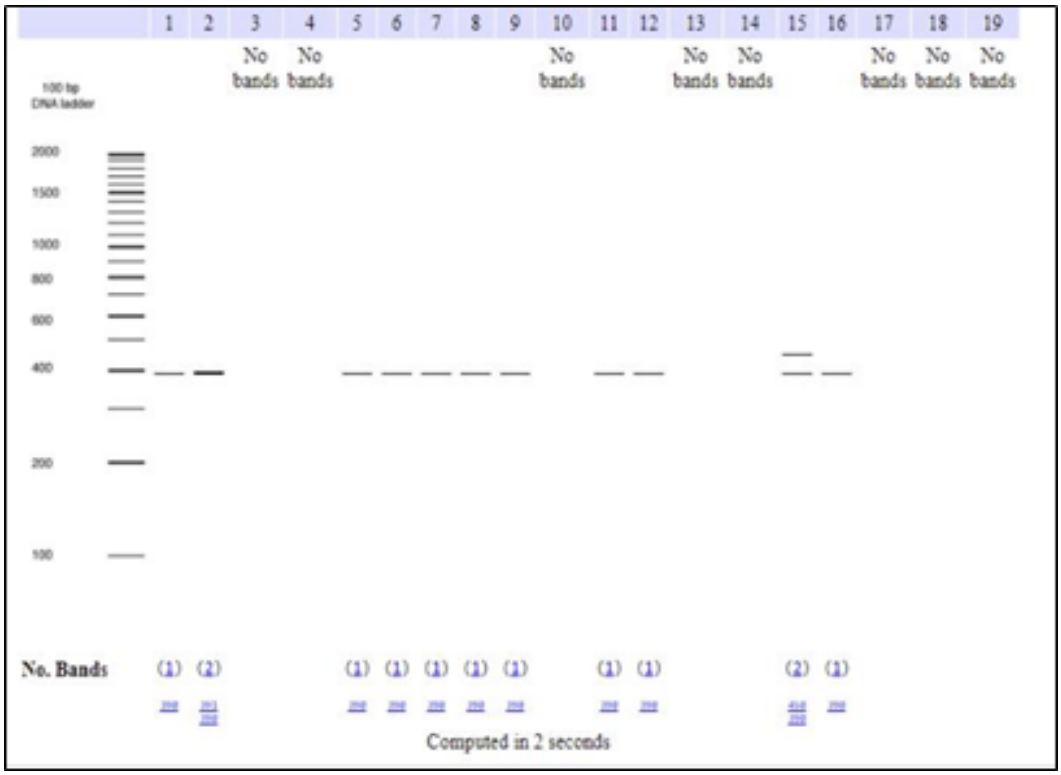

Fig. 6. Showing the positive band formation for *ISA ba-1 li*ke using in-silico PCR amplification among the strains of *A. baumannii*

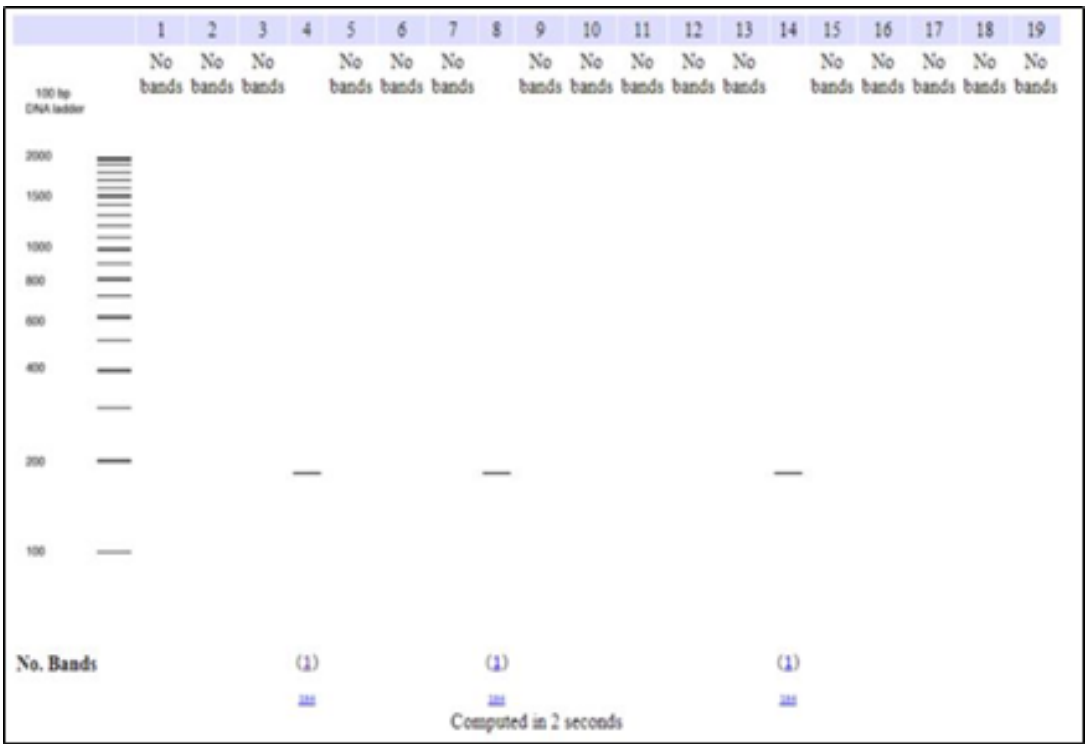

Fig. 7. Showing the positive band formation for *ISA ba-125 li*ke using in-silico PCR amplification among the strains of *A. baumannii*

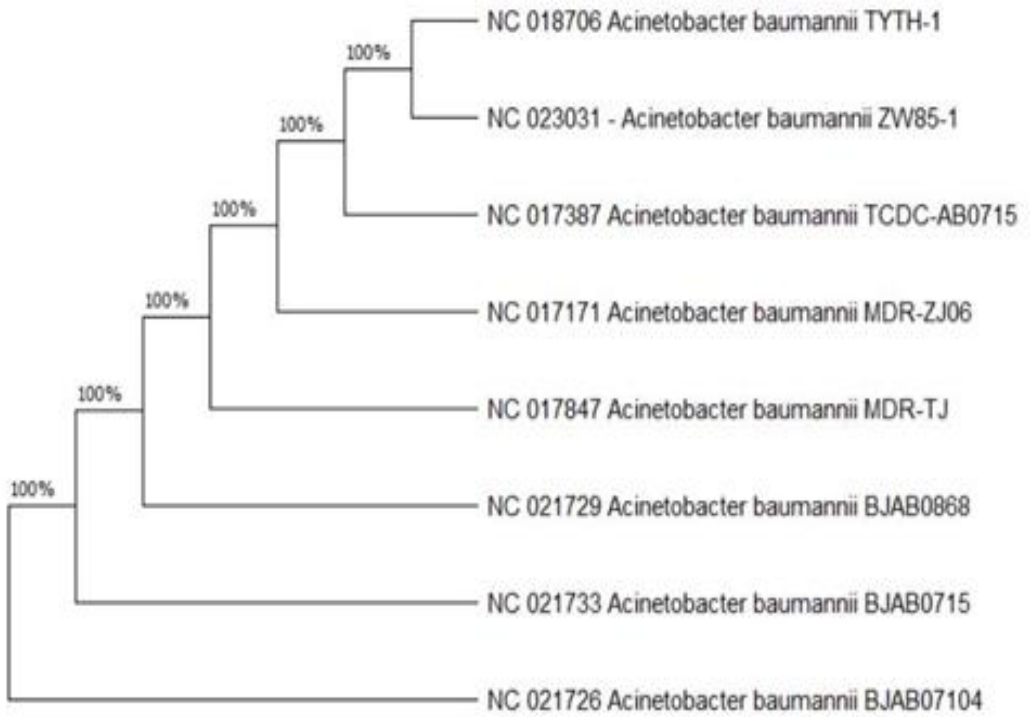

Fig. 8. Showing the positive phylogenetic tree construction for *Tet B* like using in-silico PCR Amplification among the strains of *A. baumannii*

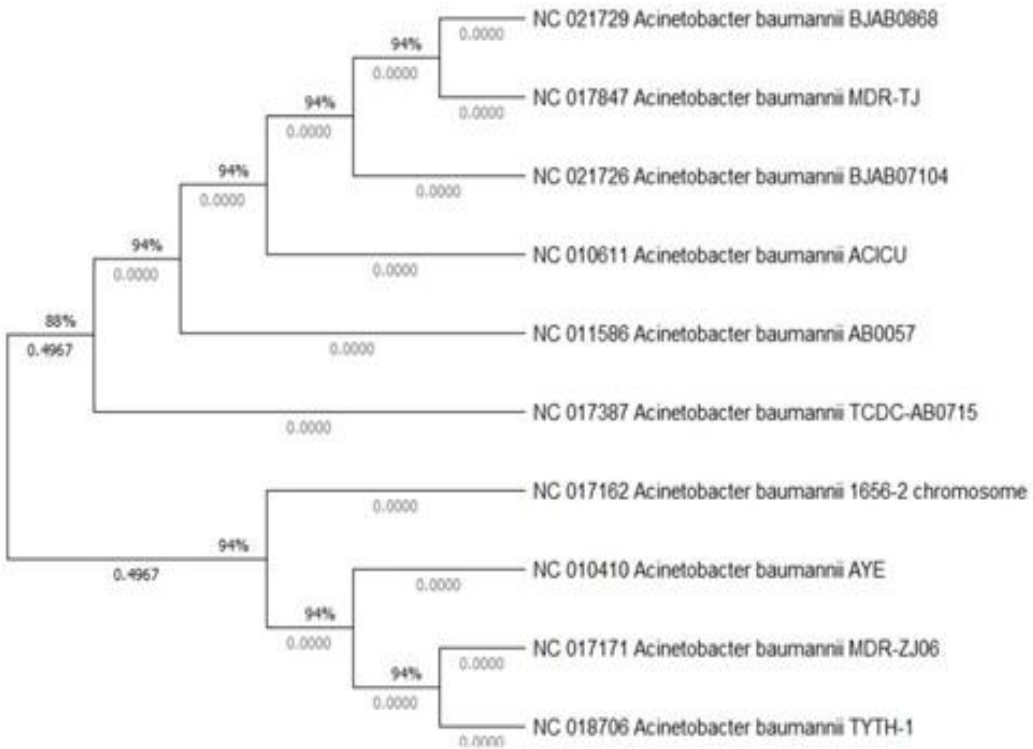

Fig. 9. Showing the positive phylogenetic tree construction for *Sul 1* like using in-silico PCR Amplification among the strains of *A. baumannii*

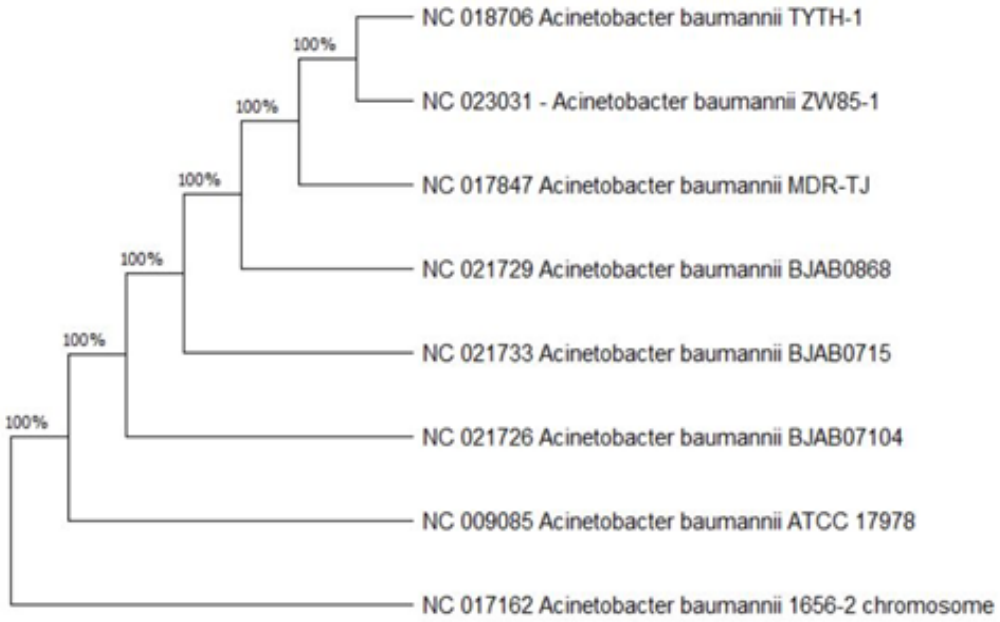

Fig. 10. Showing the positive phylogenetic tree construction for *Sul 2* like using in-silico PCR Amplification among the strains of *A.baumannii*

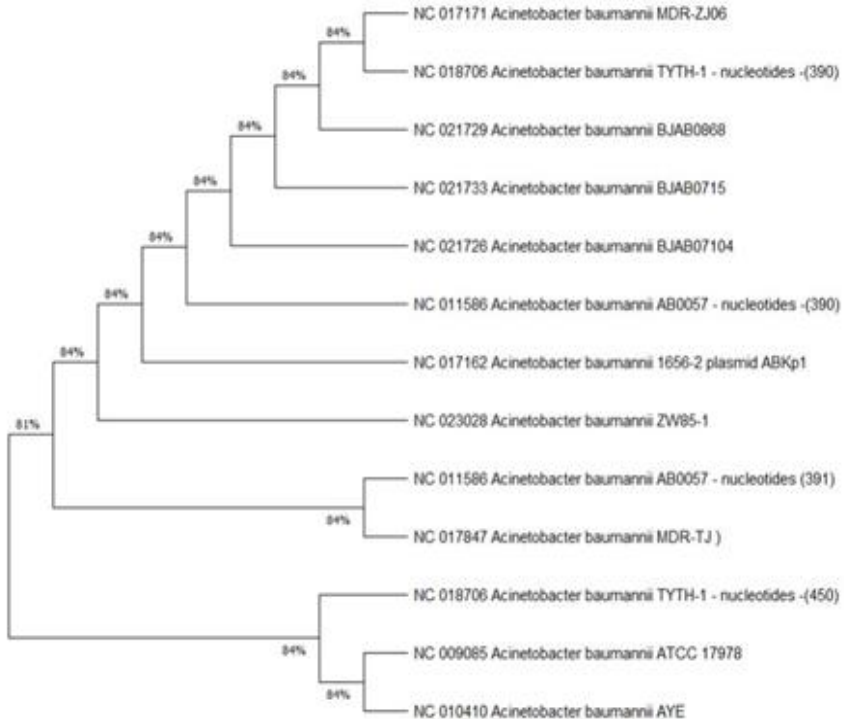

Fig. 11. Showing the positive phylogenetic tree construction for *ISAba-125* like using in-silico PCR Amplification among the strains of *A.baumannii*

4. DISCUSSION

According to a previous study, *A.baumannii* is one of the most common gram-negative bacteria that causes nosocomial infections among hospitalized patients [30]. They can live on inanimate surfaces for longer periods of time and can tolerate desiccation. Because of its

propensity for multi-drug resistance, it has spurred the attention of many researchers in recent years. Computational based approach to detect the genetic determinants can be easily achieved by designing the primers for polymerase chain reaction on an in-silico based platform using specific tools [16]. Recent technological advancements have also made it simple to change a high precise theoretical probability of a good PCR. Instead of an expensive laboratory assay, sensitive primers are used as input sequence in the servers to classify the potential outcomes of a target gene. In the same way, the present study was conducted to assess the frequency of the gene distribution among the selected strains using unique primers [31].

Higher frequency of *tet B, Sul 1, sul 2, dfr A1, ISAba-1, ISAba-125, Tet A* were observed in this study. In the present study we have attained results such that the virulence of *tet B* as 42.10%, *Sul 1* as 52.63%, *Sul 2* as 42.10%, *dfr A1* as 5.26%, ISAba-1 as 63.15%, *ISAba-125* as 15.78% and *tet A* as 10.52%. In the previous study it has shown that 50% of virulence is seen for *tet B [30,*32]. There was a study reporting that they have found 36% virulence of *qnrA* and 41% of *qnrB [16]* whereas we obtained 0% distribution of *qnrA* and *qnrB*. A similar result was obtained in a previous study where higher virulence of *ISAba-1* was found [33]. There was a similar study which concluded dfrA1 virulence to be of 6% [34].

Also there have been other studies stating that there were no higher frequencies of *tet B*, *Sul 1 Sul 2* and *ISAba-1* also they have reported that there was only a low frequency of the genetic determinants. In an early study [35], a high frequency of extended spectrum beta lactamase producing strains were observed with low frequency of other resistant genes [33]. As a result, the present investigation showed both correlating and contrasting results in comparison to earlier studies.

This indicates that in-silico PCR amplification is better suited for tentative gene recognition. In-silico based computational analysis holds promising to identify the virulence genes [23] and resistance genes in our earlier studies [24, 25]. The in-silico based analysis can also be implemented to select novel drug target from the non-antibiotic drugs as well [26]. Preliminary identification of the virulent and resistant strains can be further target by bioactive compounds from natural sources [27 – 31]. Computational platform serves its best for the viral studies as well [32, 33, 34]. The study also can be best implemented for the dental based studies [35, 36, 37]. Selection of novel compounds from marine source can also be achieved using the in-silico based tools [38].

The study was conducted with *A. baumannii* as the target strain as it is a multi-drug resistant strain [39] and can also be targeted by the natural compounds [40]. The study has its own limitation that the detection of the resistant determinants is not evaluated as an in-vitro study using the clinical strains. As a future prospect, further in-vitro trials with clinical strains are required to determine the prevalence and distribution of virulent and resistant genes among the clinical isolates of *A. baumannii*.

5. CONCLUSION

The occurrence of seven important genetic determinants of resistance was observed among the 13 genes studied in this research. The selected 19 *A. baumannii* strains had higher frequencies of *tet A, tet B, Sul 1, Sul 2, DfrA1, ISAba-1 and ISAba-125.* The in-silico PCR technique is promising at a preliminary level in identifying the genetic determinants. However, further laboratory tests using clinical isolates as well as frequent monitoring of the resistant strains for epidemiological surveillance is required to curb the *A. baumannii*-related infections.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

Institutional approval for conducting the research was prior obtained (IHEC/SDC/UG-1901/21/149).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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