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Frequency of Adhesive Virulence Factor *fimH* among the Clinical Isolates of *Acinetobacter baumannii* in India

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Authors' contributions

This work was carried out in collaboration among all authors. Author SP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASSG and JVP managed the analyses of the study. Author ASSG managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of the study was to detect the presence of *fimH* gene among the drug resistanst strains of *Acinetobacter baumannii. fimH* gene was found to be associated with a catch bond mechanism which led to better evolution of biofilm formation. Since there are not many studies done with this gene it would be a timely investigation and this study mainly aims in molecular characterization of *fimH* gene among clinical isolates of *A. baumannii*. Semi quantitative bio adherent assay was done by the multidrug resistant strains of *A. baumannii* to find the formation of biofilm. The DNA was extracted with the help of kit and PCR was performed for amplification. Pearson correlation analysis was done to find the existing correlation between the *fimH* gene and MDR strains of *A. baumannii* with significant p-value of (<0.05). From the screened 73 genomes of MDR *A. baumann*ii 6.8% showed positive amplicons for the *fimH* gene which were related to biofilm

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and porin formation (Fig. 1). Correlation of its existence was high in beta lactamase (100%), cephems (100%), folate (100%) resistant strains, followed by aminoglycosides (80%), carbapenems (60%) and fluoroquinolones (60%) and efflux pumps (20%). In Spite of various measures undertaken to prevent the disease, the prevalence of the pathogen is multiplying. The current study recorded the presence of *fimH*gene (6.8%) among the clinical isolates of *A.baumannii*. This gene can be used as a target to develop new drugs and vaccines to combat the menace of *A.baumannii* infection.

Keywords: A. baumannii; biofilm; fimH gene; antibiotic resistance.

1. INTRODUCTION

Acinetobacter baumannii is a gram negative bacilli and an opportunistic bacterial pathogen mainly related to nosocomial infection [1]. It is gaining more prominence by attracting the attention among researchers who are amazed by its tendency to resist even the last resort of antibiotics and to pave the way for many diseases which aid in increasing the mortality rate [2]. They can also quickly adapt their genome to the wavering situation [3]. In *A.baumannii*, the formation of biofilm is known to be one of the significant factors which impede the action of antibiotic drugs. They protect the bacteria from the defense mechanism of the host and help in better communication between the bacteria so that they express and explode their virulence traits [4]. Biofilm formation thus play an important role in exhibiting the pathogenicity among the *A.baumannii* strains.Amidst various biofilm associated gene operon in *A.baumannii*, *fimH* mediated fimbriae associated protein helps in gluing themselves to the cell wall leading to the creation of the so called catch bond mechanism [5]. *fimH* is also known for its frequent modification of its amino-95acid composition leading to strong initiation and evolution of biofilms [5]. *fimH* falls under the category of adhesive virulence factor, and in *A.baumannii* it is associated with various functions such as adhesion, biofilm formation and survival in harsh environments [6]. Mutations in the *fimH* gene exhibit better adhesive properties too resulting in the development of the biofilms and its typing can be used to investigate and understand the population structure of microorganisms [7]. Many studies have documented up to 95% *fimH* gene expression associated with the virulent traits in *A.baumannii* [8]. *fimH* falls under the type 1 fimbriae category of the adhesive virulence factors and *fimH* mediated adhesive virulence is to be associated with the drug resistant strains of *A.baumannii*.With this background assessment on the correlation of the prevalence of *fimH* gene among the multidrug resistant clinical isolates of *A.baumannii* would be a timely investigation as it not so vivid in many studies from South India. Thus this study is aimed to molecularly characterize *fimH* gene among the clinical isolates of *A. baumannii* with further comparative genomic assessments of the sequenced amplicons of the *fimH* gene.

2. METHODS

2.1 Detection of Biofilm Formation by Semi Quantitative Adherent Assay

The cells were obtained from drug resistant strains and the formation of biofilm were observed by culturing them [9]. The assay was carried out for every strain in 200microlitre of fresh broth culture,in soy broth, with 0.25% glucose. The plate was incubated with negative and positive control at 37 °C and the well was washed three times with phosphate buffer to remove the free cells. The bacteria were fixed using 95% ethanol. The plates were dried and all the wells were stained with 100microlitre of 1% crystal violet solution. Excess stains were removed by washing them with distilled water and then dried. Optical density was examined and biofilm formation was graded as high(OD>1), low (0.1<OD<1) or negative(OD<0.1) [10].

2.2 Extraction of Genomic DNA

Non-repetitive 73 multidrug resistant strains of*A.baumannii* as reported in our earlier studies [11] stored at -80°C in 80% / 20% (v/v) glycerol in LB medium were freshly retrieved on the Mac Conkey agar with incubation at 37°C/24 hrs. The chromosomal DNA was extracted using the Qiagen DNA extraction kit in accordance with the manufacturer's instructions. Extracted genomic DNA was stored in -20°C for future use.

2.3 PCR Amplification of *fimh*

PCR reaction mixture [15 µl] was prepared by adding 7.8 µl of 2x master mix [Taraka, Japan] in

5.6 µl of double distilled water with 0.31 µl of 100 pmol/ml concentration of the specific *fimH* primers [Eurofins Genomic India Pvt Ltd, Bangalore]. 1 µl of the DNA was added to the primary mixture and further amplification was performed with the PCR condition for 35 cycles (Fig. 1) Eppendorf thermocycler (PCR instrument), Germany. The acquired PCR amplicons were analysed in 1.5% agarose gel electrophoresis which incorporates ethidium bromide and was observed by gel documentation framework. The 100 bp DNA ladder was used as the marker to assess the size of the positive *fimH* amplicon.

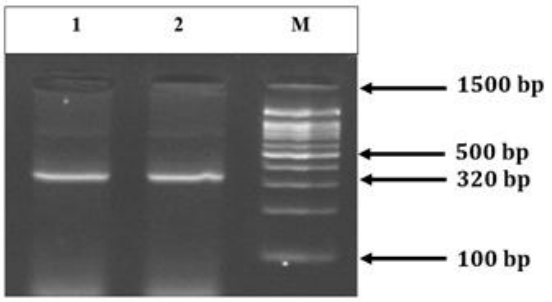

Fig. 1. Electropherogram of *fimH*gene with an amplicon size of 320bp

3. RESULTS AND DISCUSSION

3.1 Correlation of Fimh with Multidrug Resistance

Semiquantitative adherent bioassay for biofilm formation exhibited 58.9% under high grade, 31.5% under low grade, and 0.9% were detected to be negative. Among the 43 strains of high grade biofilm formers all 100% were multi drug resistant. They showed resistance against 3 classes of antibiotics when examined followed by 91.3% under low grade. Only one strain was negative when detected under low grade biofilm formers. Pearson correlation analysis showed positive results which gives the possibility of correlation existing between *fimH* gene with drug resistance strains and the p-value was found to be (<0.05).

From the screened 73 genomes of MDR *A.baumannii* 6.8% showed positive amplicons for the *fimH* gene which were related to biofilm and porin formation (Fig. 1). Correlation of its existence was high in beta lactamase, cephem, folate resistant strains, followed by aminoglycosides, carbapenem and fluoroquinolone. Control susceptible stain of *A. baumannii* yielded 9.5% in comparison with the MDR strains (Fig. 1). The graph denotes the frequency of *fim*H gene among the antibiotics (Fig. 2).

In recent years *A. baumannii* has emerged as a priority nosocomial pathogen complicating the systematic ailments of hospitalized patients and has also been ranked as the third most prevalent pathogen identified in ICU's [12]. Extent of resistance exhibited by *A. baumannii* against different classes of antibiotics results in failure of the various treatment strategies in hospital set-ups. In addition, high frequency of biofilm formation mediated by various biofilm associated genes often correlates with the Drug resistance among the clinical strains of *A. baumannii* [13]. Amongst various biofilm related genes adhesion based genetic determinants is highly attributed for the bacteria to colonize and to establish an infection in host

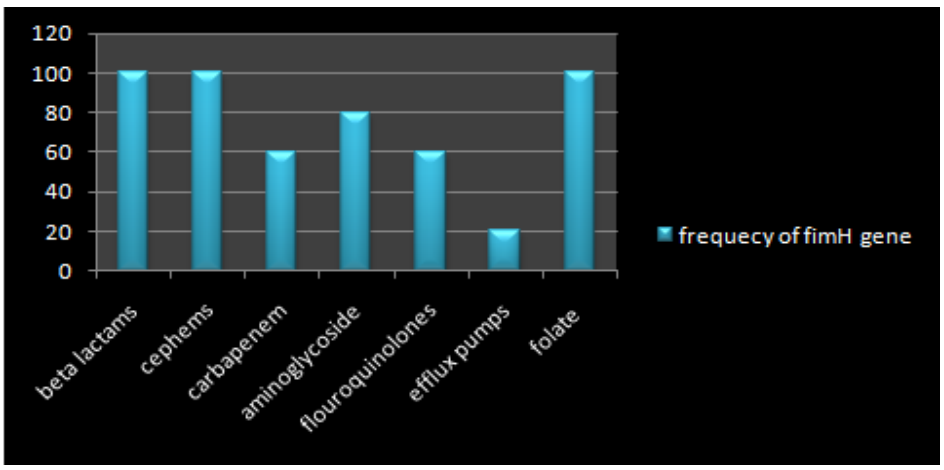

Fig. 2. Frequency of *fimH*gene among different groups of antibiotic resistant strains of *A. baumannii*

tissues. In this view, *fimH* seems to be a potent contributor for adhesion based biofilm formation on the biotic and abiotic surfaces in the varying hospital niches [14]. The present study is thus a timely assessment to observe the correlation of the occurrence of *fimH* gene among the multidrug resistant strains of *A. baumannii*. Occurrence of *fimH* gene seems to be high in various gram negative bacteria including *P.aeruginosa* and in a study from Iraq, *fimH* gene positivity was reported up to 100% and 68% among the clinical and environmental strains respectively [15]. Occurrence of *fimH* gene high in most of the earlier studies among the drug resistant clinical strains ranging from 47.38% [16] to 60% [17] prevalence. Accordingly our study had documented 6.8% of the same among our clinical isolates pertained to resistance. This correlation again substantiates the relation between biofilm based virulence associated gene and antibiotic resistance in varying hospital habitats [18]. In contrast, a study conducted by Habib et al, 2019 with the drug resistant strains of *A. baumannii* from the intensive care units showed no occurrence of *fimH*. This might be related to the *fimH* adherence which would be more in abiotic surfaces than its frequency from the clinical isolates of gram negative bacteria. Biofilm formation on the surface of the cells normally reduces the effect of antibiotics especially altering the binding sites of β-lactam group of drugs (penicillin binding site) resulting in resistance to β-lactam inhibitors and cephalosporin group of drugs. In correlation with this occurrence of *fimH* amongst beta lactamase producers was 100% in the present study. A study conducted in China, hetero-resistance among the biofilm forming *A. baumannii* strains, was observed with both cephalosporins (cefipime and ceftazidime) and penicillin resistance with 65% and 68.98% against respectively [19,20]. Frequency of *fimH* among the cycline group of drugs was 20% in the present study. *A. baumannii* exhibits resistance to cycline groups of drugs viz, tetracycline, doxycycline and minocycline through efflux pumps like RND pumps, MATE pumps etc. Biofilms attributing for adhesion on the cell surface, directly or indirectly contribute to the ejection of the cycline group of drugs out of the cell by altering the cell membrane. In view with this detection of *fimH* was observed in an earlier study with a frequency of 82.35% among all the tetracycline resistant isolates. Similarly, frequency of *fimH* was 80% in aminoglycoside resistant isolates involving the drugs viz., gentamicin, amikacin and tobramycin and it correlates with the 86% of its occurrence among the 65% gentamicin resistant strains [21]. As per the review conducted in Pennsylvania, aminoglycosides being the potent bactericidal agents are known to create fissures in the outer membrane of the bacterial cells apart from the aminoglycoside modifying enzymes [22] which again directly might get influenced by biofilm formation [23,24]. Carbapenems being considered as the last resort drugs of choice to treat various nosocomial infections caused by *A.baumannii*, resistance against the same had transformed them into a new entity called carbapenem resistant *A. baumannii*(CRAB) [23]. This group exhibits resistance against imipenem, doripenem and meropenem and in the present study occurrence of *fimH* was detected in 60%of the CRAB strains. Carbapenems being known to target the penicillin binding proteins resulting in the inhibition of the cell wall synthesis again can be influenced by the biofilm formation [25]. The prevalence of *fimH* gene in carbapenem resistant strains is yet another finding of the present investigation that occurred in a low frequency when compared to the 60% in CRAB strains in an earlier study [26,27]. *A. baumannii* exhibits resistance to drugs like co-trimoxazole/ trimethoprim sulfamethoxazole involved with folate pathway and sulphonamide resistance are involved with efflux pump and target site mutations[28]. In the present study, occurrence of *fimH* was detected up to 100% in 39 strains of aminoglycoside resistant strains employed for our study. However in an earlier study by Askari et al.2019, the prevalence of *fimH* gene was found to be 82.35% among the 70.58% of the cotrimoxazole resistant strains and according to the study by Marziyeh et al,2019 *fimH* gene was detected as 81.81% among 59.09% trimethoprim resistant isolates [29]. Similarly, in fluoroquinolone resistant isolates, *fimH* occurrence was 60% in comparison with the 58% of its frequency amidst 91% of the resistant isolates [30].

4. CONCLUSION

The present study has thus highlighted the correlation of *fimH* associated biofilm formation among different drug resistant strains of the clinical isolates of *A. baumannii*. This urges the further need for proper experimentation to detect the exact relation of *fimH* mediated biofilms in influencing the drug resistant patterns [31,32]. Also, appropriate surveillance and control

measures are essential in deducing the frequency of the same to prevent the transmission of biofilm forming MDR *A. baumannii* in a developing country like India.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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