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Genome of *Acinetobacter baumannii* Carries Multi Drug Resistance Genes

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ABSTRACT

Background: The study conducted to determine resistant and virulent genes in multi drug resistance *Acinetobacter baumannii*. **Aim**: *A. baumannii* has emerged as an important human pathogen in hospitalized patients, and leads to increased infection with significant morbidity and mortality. The study aimed to confirm the presence of resistance and virulent genes in multidrug resistance strains of *A. baumannii*. **Methods**: The multidrug resistance was screened for the several strains of *A. baumannii* and two strains Aba03 and 04 was used for screening the marker presence of gyrA, blaOXA-51, ISAba1-A, ampC-AB and strB in the genome using polymerase chain reaction. **Results and Discussion**: *A. baumannii* found to be positive for the marker genes gyrA, blaOXA-51, ISAba1-A and ampC-AB and negative for strB gene in both the strains. **Conclusion**: This report highlights the functions of novel resistant and virulent determinants, in bringing drug resistance in *A. baumannii.*

KEYWORDS: *Acinetobacter baumannii;* Drug resistance; Marker genes; PCR.

INTRODUCTION

Acinetobacter baumannii is a well known emerging pathogen, causing hospital-acquired infections worldwide[1]. Being able to survive for longer periods in hospital sites and also capable of causing serious hospital outbreaks, clinically significant *A. baumannii* acquired multidrug resistance and consequently reduces the therapeutic options[2]. In most hospital settings, ventilator-associated pneumonia is increasingly linked with nosocomial pneumonia in intensive care units [3, 4]. In recent time, many studies have reported the danger of resistance acquiring *A. baumannii* and have particularly focused on those showing multidrug-resistant strains. The cause of acquisition of MDRAB is related to multiple factors, including contact with transiently colonized healthcare providers and environmental contamination[5].

Currently, several strains of *A. baumannii* are highly resistant to the majority antibiotics available in clinical practice. A number of resistance mechanisms to many classes of antibiotics including ß-lactamases, multidrug efflux pumps, aminoglycoside modifying enzymes, permeability defects, and the alteration of target sites are known to exist in *A. baumannii* [6-8]. Most of these resistance mechanisms can effectively target different classes of antibiotics. However, combination of mechanisms can work together to contribute to the resistance to a single class of antibiotics [9]. In addition to ß-lactamases having carbapenem hydrolyzing activity as a major carbapenem resistance mechanism, which include metallo-ß-lactamases (MBLs) and carbapenem-hydrolyzing class D ß-lactamases (CHDLs), porins such as CarO [10] and penicillin-binding protein alteration might also be involved in carbapenem resistance [11]. The multidrug-resistance determinants acquired in *A. baumannii* is mostly through the transposon acquisition, plasmid conjugation and integron mobilization to receive clusters of genes encoding resistance to several antibiotic families [12]. Moreover, the functional insertion sequences are significant in amplifying antimicrobial resistance and gene plasticity [13, 14]. In the study the *A. baumannii* showed multi drug resistant have been confirmed for the prevalence of the genome linked resistant and virulent genes brining such pathogenicity.

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MATERIALS AND METHODS

Medical Samples

A. baumannii were isolated from the patient's samples such as pus, urine, blood, vaginal semen, endo tracheal secretion, plural secretion, sputum, bronchial secretion, surgical pus, abscess, drain fluid, cerebrospinal fluid, burn wound and accidental wound.

Gene Targeting

The genomes of *A. baumannii* strains Aba03 and Aba04 were selected for the screening of resistant and virulent genes presence us-

ing PCR based amplification. Five genes which have been reported to be involved in pathogenicity were selected named as gyrA, blaOXA-51, ISAba1-A, strB and ampC-AB. The primers sets and PCR conditions used were given as:

All PCR contained 12.5 µl of 2× Master Mix (Ampliqon, Denmark), including 1× PCR buffer, 1.5 mmol/l MgCl2, dNTPs at a concentration of 0.15 mmol/l each dNTP, 1.25 U of Taq DNA polymerase, 0.5 µl of 0.8 µM of each primer, 1 µl of template DNA (50 ng), and sterile distilled made up to 25 µl.

1) GyrA gene[15]

Template: Genomic DNA: Cycle conditions – 95°C for 1min., 35 cycles - 95°C for 30 sec, 52°C for 30 sec and 72°C for 2 min final extension at 72 °C for 10 min.

2) bla OXA51[16,17]

Template: Genomic DNA: Cycle conditions -94°C for 3 min followed by 35 cycles 94°C for 45 sec, 57°C for 45 sec, 72°C for 1 min, Final extension at 72°C for 5 min.

3) ISAba1[18]

Template: Genomic DNA: Cycle conditions - 94°C for 2 min followed by 35 cycles, 94°C for 10 sec, 50°C for 10 sec, 72°C for 130s , Final extension at 72°C for 1min.

4) StrB[19]

Template: Genomic DNA: Cycle conditions by Gradient PCR: 94°C for 5 min followed by 35 cycles, 94°C for 1min, 50 – 60°C for 45 sec, 72°C for 45s, and Final extension at 72°C for 10min.

5) blaampC [20]

Template: Genomic DNA: Cycle conditions - 94°C for 2 min followed by 35 cycles 94°C for 10 sec, 50°C for 10 sec, 72°C for 130s, and Final extension at 72°C for 1min.

The primers sets used for the amplification was given as:

Gene Name	Primer Sequence	Reference
gyrA gene(Fwd)	AAATCTGCCCGTGTCGTTGGT	15
gyrA gene (Rev)	GCCATACCTACGGCGATACC	
blaOXA-51(Fwd)	TAATGCTTTGATCGGCCTTG	16,17
blaOXA-51 (Rev)	TGGATTGCACTTCATCTTGG	
ISAba1-A (Fwd)	GTGCTTTGCGCTCATCATGC	18
ISAba1-A (Rev)	CATGTAAACCAATGCTCACC	
strB (Fwd)	ATGGGGTTGATGTTCATGCCGC	19
strB (Rev)	CTAGTATGACGTCTGTCGCAC	
Bla ampC-AB (Fwd)	TATGATGTGCCAGGTATGGC	20
Bla ampC-AB (Rev)	AAACTCTTCCCAACCAAGCG	

RESULTS

Resistance and Virulent Gene

In an order to determine the presence of resistant and virulent genes on the genomic location, carried PCR for strains Aba03 and 04 showed positive response to four genes namely gyrA, blaOXA-51, ISAba1-A and ampC-AB with prominent single band and negative reaction for strB (not shown) when resolved on 1.2% agarose gel (Fig. 1). The prevalence of resistant as well as virulent genes on the same genome suggested that both strains available in central India inheriting these genes to the next generation and probably became stable during evolution resultant bringing antibiotic resistance.

Fig. 1a: Positive PCR for the blaOXA51 gene with *A. baumannii* strains Aba03 and 04

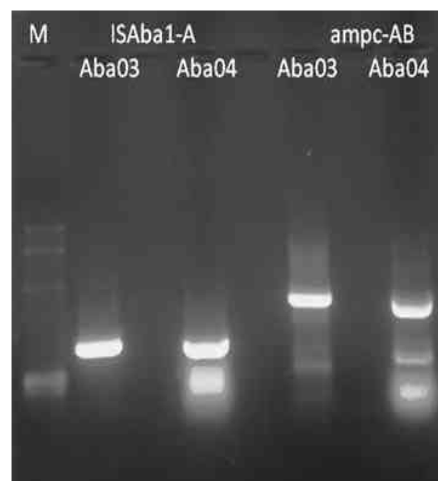

Fig. 1b: Positive PCR for the ISAba1-A element and ampc-AB with *A. baumannii* strains Aba03 and 04

Fig. 1c: Positive PCR for the GyrA gene with *A. baumannii* strains Aba03 and 04

DISCUSSION

A. baumannii is now recognized as a major pathogen generally been acquired from the hospital settings. In a study it has been recorded

that multi drug resistant *A. baumannii* were present in the human medical samples possessing resistant and virulent genes encoded by genome. The PCR based amplification confirmed the presence of four major genes viz., gyrA, blaOXA-51, ISAba1-A and ampC-AB in the multidrug resistant strains genomes. In presence of these genes, strB gene could not be ascertained which may be due to possibility of absence of such gene.

Recently many reports have also noted the presence of gyrA gene with mutation at the protein level, increasing fluoroquinolone resistance in *A. baumannii* [21, 22]. The gyrA gene also reported with mutation to cause nalidixic acid resistance [23]. Increasing resistance to carbapenems has been linked with the blaOXA gene which has also been reported to be present in the resistant Aba03 and 04 strains. Similar reports were given by the researchers where blaOXA-23-like, blaOXA-58-like, blaOXA-40-like, and ISAba1-blaOXA-51-like were identified in several *A. baumannii* isolates. Not only that, distinct blaOXA-23-like-carrying *A. baumannii* ST381 shifts the previously available blaOXA-58-like-carrying *A. baumannii* ST92/ST208, resulting in the rapidly increasing resistance to carbapenems in *A. baumannii*[24]. Along with the bacterial genome, blaOXA-23 gene found in transferable plasmid in both PFGE type 1 and 2 with emergence of ST236 in Saudi Arabia and Egypt, and ultimately spread of distinct carbapenem resistant *A. baumannii* clones belonging to ST884, ST945 and ST1096 [25].

In a study, the ISAba-1 as an insertion sequence was identified on both the strains Aba03 and04 along with resistant genes witnessed the prevailing pathogenicity of the *A. baumannii*. Presence of ISAba1-like sequences were identified immediately upstream of the bla(ampC) gene in ceftazidime-resistant *A. baumannii* isolates, but were absent in ceftazidime-susceptible *A. baumannii* isolates demonstrated the importance of the ISAba-1 presence in the strains those have shown drug resistance [26]. Insertion sequences (IS) recognized as smallest and most abundant transposable elements having ability of transposition in microbial genomes[27]. They involve in insertion mutations and genome rearrangements increases the chances of resistance and virulent determinants within species [28-32]. IS*Aba1* has been marked in *A.baumannii*, a bacterium causing nosocomial outbreaks and increasing multidrug resistance phenotype [26, 33, 34]. IS*Aba1* has linked in association with number of antibiotic resistance genes in *A. baumannii* [11, 13, 35, 36]. The function of IS*Aba1* in the expression of the antibiotic resistance gene of *A. baumannii* has been reported for *blaampC*, encoding the cephalosporinase, and for the *bla*OXA-23 gene, encoding a carbapenem-hydrolyzing oxacillinase, but it may act similarly with other resistance genes [18, 37]. IS*Aba1* probably be responsible for *bla*OXA-23 mobility, with two copies bracketing this ß-lactamase gene and forming a composite transposon (defined as Tn*2006*) [18].

Another important gene localized on the chromosome AmpC ß-lactamases are noted as clinically important cephalosporinases expressed by the chromosomes of Enterobacteriaceae and a few other organisms also. AmpC enzymes encoded by both chromosomal and plasmid genes are involved in hydrolyze broad-spectrum cephalosporins more efficiently bringing resistance to the cefotaxime, ceftazidime, and ceftriaxone [38].

CONCLUSION

A. baumannii strain continues to become a well known pathogen by bringing several genetic changes in the genome in the form of resistant and virulent genes. The co presence of these genes in the genome makes these strains more virulent and in coming time monitoring and control by using combine therapy will proved to be useful.

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