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Carbapenem Resistance Gene (OXA) Associated with IS Element in Clinical Isolates of *Acinetobacter baumannii*

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ABSTRACT

Acinetobacter baumannii is important nosocomial pathogens that are becoming more resistant to many antibiotics including Carbapenems. *A.baumannii* is mainly attributed to OXA carbapenemases and their expression may be enhanced by insertion sequence IS*Aba1* that provides promoter activity. Hence, the purpose of the study is to assess the association of different OXA carbapenmase genes with IS*Aba 1* and their effect on carbapenem resistance. A total of 75 isolates of *Acinetobacter spp.* were obtained from various clinical specimens. Antibiotic sensitivity testing was done and all the isolates were subjected to multiplex PCR for the detection OXA carbapenemase genes ($bla_{\text{OXA-23like}}$, $bla_{\text{OXA-24like}}$, $bla_{\text{OXA-51like}}$ and $bla_{\text{OXA-58like}}$). PCR mapping was performed with different primer combinations to find the association of IS*Aba 1* with the OXA carbapenemase genes. Antibiotic sensitivity pattern showed 16% of the isolates were resistant to imipenem and 17% were resistant to meropenem.23% isolates were positive for $bla_{\text{OXA-51like}}$ gene. 15% isolates were also positive for $bla_{\text{OXA-23like}}$. 5/75 isolates that were positive for both $bla_{\text{OXA-51like}}$, $bla_{\text{OXA-23like}}$ and had a copy of IS*Aba 1* upstream to the Carbapenemase gene. The results of our study show that the insertion sequence *ISAba-1* is specifically associated with the acquired carbapenemase, $bla_{\text{OXA-23like}}$ gene which might contribute to carbapenem resistance in *A.baumannii* among our isolates.

Keywords: *Acinetobacter baumannii*, Carbapenemase, IS*Aba*1

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INTRODUCTION

Acinetobacter baumannii is an important nosocomial pathogen. In recent days, the isolates are becoming more resistant to many antibiotics including carbapenem which is used in the treatment as last choice [1]. The carbapenemases are the most frequently hydrolyzing class D-β- lactamases. The major subgroups of carbapenemases that have been identified in *A.baumannii* are the naturally occurring OXA-51/69-type β- lactamase and the acquired OXA-23, OXA-24 and OXA-58-type β- lactamase [2].

The target sequence was identified in *Acinetobacter baumannii*, a 9bp direct repeats of flanking region with 11 bp inverted repeat sequences (IRs) insertion sequence. It was shown to encode an enzyme transposase and also play a major role in expression of resistant genes through promoters [1]. IS*Aba*-1 is a potential candidate belongs to IS4 family, has been found upstream of resistant genes. Hence, the present study analyzed the prevalence of IS*Aba-1* in *Acinetobacter baumannii* isolates in relation to its carbapenem resistant genes.

MATERIALS AND METHODS

A total of 75 isolates of Acinetobacter species, obtained from various specimens such as blood, pus, wound swab, sputum, endotracheal aspirate etc, were included in the study (Table 1). All isolates were confirmed using standard biochemical tests. Antibiotic susceptibility testing was done (CLSI 2013) [4]. Isolates of *A.baumannii* resistant to meropenem by disc diffusion test were subjected to MIC determination by E-test using E-strip procured from Hi-Media, Mumbai. *Pseudomonas aeruginosa* ATCC 27853 strain was used as control.

Phenotypic detection of carbapenemase enzyme:

The imipenem and meropenem resistant isolates (n=13) were subjected to modified Hodge test using Muller Hinton Agar. The ATCC 25922 *Escherichia coli* was inoculated on the agar and placed it undisturbed for 3-5minutes, after 5 min Meropenem disc was placed at the centre of the agar, and the test strain was streaked from the edge of the disc to the periphery of the agar plate. The plates were incubated for 18-24 hours and the presence of clover leaf shaped zone was noted as positive (CLSI 2013) [4].

Detection of carbapenemase gene by PCR:

Multiplex PCR to detect carbapenemase genes (bla_{OXA-23}, bla_{OXA-51}, bla_{OXA-24}, bla_{OXA-58}) was carried out with minor modifications in annealing temperature. The primers and conditions used are given in Table 2 [3]. All the 75 isolates were subjected to PCR irrespective of their resistant profile.

Table 2: Primer sequence for multiplex OXA gene

Primer	Sequence	Product size
bla_{OXA-23}-likeF	5'GAT CGG ATT GGA GAA CCA GA 3'	
bla_{OXA-23}-likeR	5' ATT TCT GAC CGC ATT TCC AT 3'	501 bp
bla_{OXA-24}-likeF	5' GGT TAG TTG GCC CCC TTA AA 3'	
bla_{OXA-24}-likeR	5' AGT TGA GCG AAA AGG GGA TT 3'	246 bp
bla_{OXA-51}-likeF	5' TAA TGC TTT GAT CGG CCT TG 3'	
bla_{OXA-51}-likeR	5' TGG ATT GCA CTT CAT CTT GG 3'	353 bp
bla_{OXA-58}-likeF	5' AAG TAT TGG GGC TTG TGC TG 3'	
bla_{OXA-58}-likeR	5' CCC CTC TGC GCT CTA CAT AC 3'	599 bp

Detection of IS element:

PCR was performed to detect the IS element IS*Aba-1, t*he primers were given in Table 3. PCR products were resolved in 1% Agarose gel electrophoresis [6]. To confirm the presence of IS element to the upstream of the resistant genes; the PCR was performed for the positive isolates of IS*Aba*-1 gene using two sets of primer. The forward primer of IS*Aba*-1 gene, the reverse primer of bla_{OXA-51}-like gene and the forward primer of IS*Aba*-

1 gene and reverse primer of bla_{OXA-23}-like gene with 1.2kb and 1.6kb of the amplified product respectively. The amplified products were then resolved on to 1.2% Agarose gel and 1kb ladder was used as marker.

Table 3: Primer sequence for IS*Aba*1 gene

Primer	Sequence	Product size
IS*Aba-1*F'	5'CAC GAA TGC AGA AGT TG 3'	549 bp
IS*Aba-1*R'	5'CGA CGA ATA CTA TGA CAC3'	

RESULTS

Seventy five isolates of Acinetobacter species were obtained from various sources of clinical specimens, the distribution were given in Table 1. The AST results were given in Table 4. 72% (54/75) of isolates showed sensitivity to imipenem. 50% of isolates showed sensitivity to gentamicin and ciprofloxacin. 33% (25/75) were resistant to carbapenems of which, 16% (12/75) and 17% (13/75) were resistant to imipenem and meropenem respectively.9.3% (7/75) isolates showed resistance to both imipenem and meropenem.

Table 1: Shows the source of Acinetobacter isolates

Source of isolate	No. of isolate (n=75)
Blood	13
Urine	10
Pus	21
Sputum	21
Wound swab	7
Endotracheal aspirate	2
Vaginal swab	1

Table 4: Shows the percentage of antibiotic sensitivity test

Antibiotics	%Sensitivity(n=75)	%Intermediate(n=75)	%Resistant(n=75)
amikacin (30µg)	55 (41)	12(9)	33(25)
ampicillin/sulbactum (10/10µg)	27(20)	12(9)	61(46)
ceftazidime (30µg)	31(23)	24(18)	45(34)
ciprofloxacin (5µg)	51(38)	12(9)	37(28)
doxycyclide hydrochloride (30µg)	47(35)	12(9)	41(31)
gentamicin (10µg)	48(36)	13(10)	39(29)
imipenem (10µg)	72(54)	12(9)	16(12)
meropenem (10µg)	70(52)	13(10)	17(13)
piperacillin (100µg)	57(43)	-	43(32)
piperacillin/tazobactum (100/10µg)	65(49)	-	35(26)
tobramycin (10µg)	59(44)	12(9)	29(22)

11/13 isolates showed the minimum inhibitory concentration at >32µg/ml, 1 isolate showed breakpoint at 1µg/ml and 1 at 4µg/ml by E strip method.

Phenotypic detection

None of the 13 carbapenem resistant isolates were positive for phenotypic expression of carbapenemase gene by modified Hodge test.

Multiplex PCR for carbapenemase gene detection

All the 75 isolates were tested for the presence of carbapenemase genes by multiplex PCR, 17 showed the positivity for bla_{OXA51-} like- and 11 isolates showed the presence of bla_{OXA-23}-like (Figure 2), only 1isolate of each was positive forbla_{OXA-24}-like and bla_{OXA-58}-like.10/75 isolates showed the presence of both bla_{OXA51}-like- and bla_{OXA-23}-like genes.

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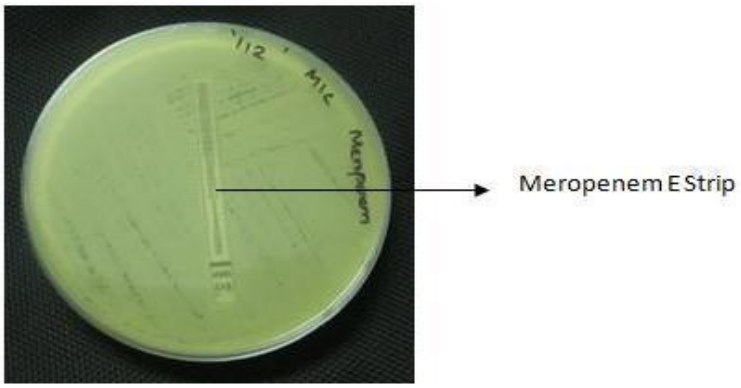
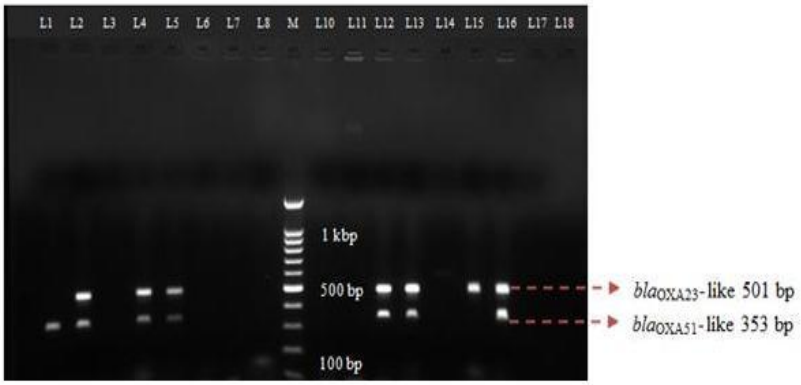


Figure 1: Shows the E test of meropenem resistant isolate

Figure 2 represents the gel picture for multiplex PCR



Lane: 2, 4, 5, 12, 13, 16 showed positive for both bla_{OXA23}-like and bla_{OXA51}-like
Lane: 1 showed positive for bla_{OXA51}-like
Lane: 14 showed positive for bla_{OXA23}-like

Detection of IS element

Table 5 summarizes the results of IS element positivity.13/75 isolates were positive for the presence of Insertion sequence IS*Aba*-1.When Touchdown PCR was carried out for the bla_{OXA-51} and IS*Aba*-1 positive isolates (i.e 13 isolates),to note that none of the isolates were shown the presence of IS element as the promoter region to the bla_{OXA-51}-like gene. 10 isolates showed the presence of 1.6kbp band which is positive for the forward primer of *ISAba-1* gene and reverse primer of bla_{OXA-23}-like gene (Figure 3). Hence it is confirmed that to express bla_{OXA-23}-like gene, IS element was acting as the Promoter region.

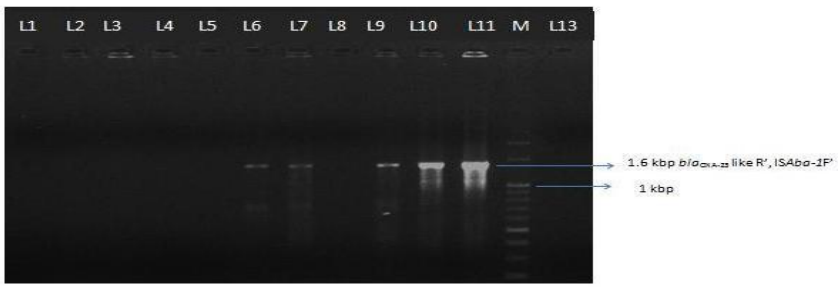


Figure 3: Shows the representative gel picture of IS*Aba-1*F' and bla_{OXA-23} like R'

Lane: 6, 7, 9, 10, 11 showed band at 1.6kbp
M: 1kb ladder

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Table 5: Summarizes the MIC and PCR results

MIC for meropenem (n=13)			Multiplex PCR for carbapenemase gene (n=75)					IS*Aba-1* gene (n=75)	bla_{OXA51}-like and IS*Aba-1* (n=13)	bla_{OXA-23} like and IS*Aba-1* (n=12)
≥32µg/ml	4µgm/l	1µg/ml	bla_{OXA51}-like	bla_{OXA-23} like	bla_{OXA-24} like	bla_{OXA-58} like	bla_{OXA-23} like and $bla_{OXA51-like}$			
11	1	1	13	12	1	1	13	13	0	10

DISCUSSION

It has been very well established by different studies that *Acinetobacter baumannii* was found to carry mixed resistance genes which imparts the organisms to get more resistance towards broad spectrum antibiotics [7]. IS elements are frequently transferred among bacteria on transmissible plasmids, which were present in *Acinetobacter spp.* The abundance of this IS elements favors the occurrence of transposition event frequently [5]. Previous studies, had reported that different *ISAba-1* promoter sequences are at the upstream of the start site of the resistance genes [3]. In the present study, the minimum inhibitory concentration for meropenem was more than 32µg/ml for those strains positive to both bla_{OXA-51} and bla_{OXA-23}. IS*Aba*-1 was detected in most of the strains which was positive for the carbapenemase gene bla_{OXA-23}. The finding of our study was also well correlated with earlier studies, that ISAba1 possessing isolates was associated with bla_{OXA-23} and not with bla_{OXA-51} gene [5]. PCR was carried out to study the presence of IS*Aba*-1 as promoter for the sole carbapenemase gene, in this regard we found that all the isolates tested for IS*Aba*-1 F' and bla_{OXA-23} R' were shown positive whereas, none of the isolates showed positive for the presence of promoter to overexpress the bla_{OXA-51} carbapenemase gene.

But in a recent study, the isolates with higher rates of imipenem resistant plasmids bearing IS*Aba*1-bla_{OXA51}-like gene did not contain any other OXA encoding genes [8]. Isolates with bla_{OXA-23} gene were consistently resistant to imipenem and meropenem with MIC value of ≥32µg/ml, and isolates with bla_{OXA-51} was susceptible to carbapenems [5]. It is remarkable to note that in our study also, all the meropenem resistant isolates showed positive for the presence of promoter to express the bla_{OXA-23} carbapenemase gene. And about all the resistant isolates were bearing both bla_{OXA-51} and bla_{OXA-23} genes. These isolates were resistant to meropenem and imipenem by AST and showed minimum inhibitory concentration of >32µg/ml. The isolates which showed positivity only for bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51} and bla_{OXA-58} genes were negative for the presence of IS elements and moreover, those isolates were sensitive to carbapenem drugs.

CONCLUSION

Hence, the study showed the insertion elements IS*Aba*-1, acts as the promoter and may lead to the over expression of carbapenemase OXA gene [9]. From the study we could conclude that all the isolates which carry OXA gene and the insertion elements were also resistant to carbapenem group of drugs by MIC. The detection of the presence of IS elements in *A.baumannii* may give the organism more flexibility to acquire the resistance easily and make the organism more pathogenic.

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