Prevalence of *ESBL* Encoding Genes in *Acinetobacter baumannii* Strains Isolated from Various Samples of a Tertiary Care Hospital in Mymensingh

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Abstract

The aim of this study was to find the prevalence of *ESBL* genes among *A. baumannii* isolates. In this cross sectional study, 49 *Acinetobacter* spp. were isolated from various clinical samples from March 2019 to February 2020 conducted in the Department of Microbiology Mymensingh Medical College. Clinical samples including endotracheal aspirates, wound swab/pus, urine and blood. A total of 380 samples were analyzed. Growth was obtained in 34.21% of the samples yielding 130 organisms. Out of 130 organisms, 49 (37.69%) were *Acinetobacter* spp. Among 49 *Acinetobacter* spp, 39 (79.59%) were *Acinetobacter baumannii* which was identified by PCR targeting *OXA-51* like gene. Amplification of the *ESBL* encoding genes, namely *CTX-M*, *TEM*, *SHV* done by molecular technique PCR. The most antibacterial resistance was against ceftriaxone (79.48%) and lower resistance only showed in colistin (12.82%). All the isolates were sensitive to tigecycline. The distribution of *ESBL*s genes such as *TEM* 20 (51.28%), *CTX-M* 16 (41.02%) and *SHV* 0 (0%). The high resistance to most of the antibiotics among the studied strains and also a high prevalence of *TEM* gene in *A. baumannii* strains found in our study gives alarming sign towards the treatment complexity of these strains.

Keywords: *Acinetobacter baumannii*; Antimicrobial resistance; extended-spectrum-β-lactamase

Introduction

Acinetobacter baumannii is an opportunistic pathogen, with the following characteristics of being Gram-negative, oxidize-negative, non-fermentative, non-motile coccobacilli and has broad range of antibiotic resistance. High morbidity and mortality are the characteristics of nosocomial infections caused by *Acinetobacter* spp., which included urinary tract, skin and soft tissue infections, pneumonia and bacteremia especially in patients with severe health condition [1-3]. The antimicrobial resistance in this nosocomial pathogen is mainly caused by β-lactamase inactivating enzymes, alteration of membrane porin channels and mutation that changes cellular function [4]. Widespread dissemination of *A. baumannii* infections, which are resistant to β-lactam antibiotics particularly to the 3rd generation of cephalosporin's and carbapenems, has become a globally significant problem [5]. Extended- Spectrum β-Lactamases (*ESBLs*) are enzymes that confer resistance to penicillin's, 3rd generation cephalosporin's, monobactams and determined by *CTX-M*, *TEM* and *SHV* genes [6]. These enzymes are mainly plasmid mediated and most are parts of the *TEM* and *SHV* families [5,7]. *ESBLs* play an important role in resistance against later generation cephalosporin such as cefepime, Cefotaxime and ceftazidime [8]. Concerning the emergent importance of *ESBLs* in antibiotic resistance and its effect on treatment failure, this study was led to assess antibiotic sensitivity patterns of studied strains and determine the prevalence of *CTX-M*, *TEM* and *SHV* genes in *A. baumannii* strains isolated from tertiary care hospital in Mymensingh.

Materials and Methods

This cross sectional observational type of study was conducted in the Department of Microbiology of Mymensingh Medical College (MMC) over a period of 1 year from March 2019 to February 2020 after getting approval from Institutional Review Board (IRB) of MMC. Written consent was taken from all the participants of this study. Patient who were admitted in the ICU, Surgery and Burn unit of MMCH with devices, wound infection and sepsis were included in this study. All clinical specimens were routinely cultured in MacConkey and Blood agar media. Typical colonies were enumerated, picked and examined further. *Acinetobacter* spp. was identified by gram-

staining, colony morphology, positive catalase, and negative oxidase test, absence of motility and fermentation tests [9]. *A. baumannii* was confirmed by detecting *OXA-51* like gene [10]. Finally, all the isolates were evaluated with the PCR technique to detect the *ESBL* [11] genes of interest. The confirmed isolates were kept frozen -80°C for further tests.

Antibiogram

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar by Kirby-Bauer disc diffusion method [12]. The tested antibiotics were: Amikacin (30 µg), Gentamicin (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Doxycycline (30 µg), Cotrimoxazole (1.25/23.75 µg), Piperacillin-Tazobactam (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Imepenem (10 µg), Meropenem (10 µg), Colistin (10 µg) and Tigecycline (15 µg). The antibiotic discs were obtained from Mast CO, UK. Zone diameter interpreted by according to (CLSI-2018) guidelines [13] except tigecycline (FDA approved) [14]. *Pseudomonas aeruginosa* ATCC 27853 was used as control strain for antibiotic sensitivity.

Amplification of *OXA-51* like gene

The bacterial DNA was extracted by boiling method [15]. The specific primers including *OXA-51* (Table 1) were used for PCR amplification of the genes [10]. PCR amplification procedure was performed using 25 µl master mix containing DNase free water 18.25 µl, buffer 2.5 µl, dNTP 2 µl, Taq polymerase 0.25 µl, primer 1 µl and DNA 1 µl. PCR amplification was done in thermal cycler device. Agarose gel electrophoresis of the amplified DNA product with 100 bp size marker was carried out in a 2% agarose gel for 40 minutes at 80 V and stained with ethidium bromide to detect 353 bp bands.

Amplification of *ESBL* genes

The specific primers including *CTX-M*, *TEM* and *SHV* (Table1) were used for PCR amplification of the genes [11]. PCR amplification procedure was performed using 25 µl master mix containing DNase free water 18.25 µl, buffer 2.5 µl, dNTP 2 µl, Taq polymerase 0.25 µl, primer 1 µl and DNA 1 µl. PCR amplification was done in thermal cycler device. Agarose gel electrophoresis of the amplified DNA product with 100 bp size markers was carried out in a 2% agarose gel for 40 min at 80 V and stained with ethidium bromide.

Results

A total of 380 samples were analyzed which included tracheal aspirates (70), wound swab/pus (175), catheterized urine (70) and Blood (65). Growth was obtained in 34.21% of the samples yielding 130 organisms. Majority were isolated from endotracheal aspirates (Table 2).

Table 3 showing among 130 isolated strains, 49 *Acinetobacter* spp. (37.69%) were isolated. Majority were isolated from endotracheal aspirates. Table 4 showing among 49 *Acinetobacter* spp. 39 (79.59%) were *Acinetobacter baumannii* which was identified by PCR targeting *OXA-51* like gene and 10 (20.41%) were other than *Acinetobacter baumannii*. Table 5 showing *Acinetobacter baumannii* was highly resistant (>50%) to most of the antibiotics and lower resistance only showed in Colistin (10.20%). All the isolates were sensitive to Tigecycline. Table 6 showing among 3 *ESBL*s genes, *TEM* (51.28%) and *CTX-M* (41.02%) were predominant respectively. Strains for *SHV* genes were not detected.

PCR was done to detect the *OXA-51* like gene for *Acinetobacter baumannii*, Lane 1, 2, 3, 4, 5, 7, 8, 9, 10, and 11 are showing bands of

Table 1: Primers used for PCR amplification of the studied genes.

Primer name	Primer sequence	Product size (bp)	Reference
OXA-51	TAATGCTTTGATCGGCCTTG	353	10
	TGGATTGCACTTCATCTTGG		
CTX-M	ATGTGCAGTACCAGTAAGCGTCATGGC	593	11
	TGGGTAAAATATGTCACCAGAACCAG		
TEM	TCGCCGCATACACTATTCTCAGAATGA	445	11
	ACGCTCACCGGCTCCAGATTTA		
SHV	ATGCGTTATATTCGCCTGT	747	11
	TGCTTTGTTATTCGGGCCAA		

Table 2: Isolated organisms from different samples (N=380).

Types of samples	Number	Percentage (%)
Endotracheal aspirate	70	68 (97.14)
Wound swab	175	30 (17.14)
Catheterized urine	70	27 (38.6)
Blood	65	5 (7.7)
Total	380	130 (34.21)

Table 3: Distribution of *Acinetobacter* spp. isolated from different samples (n=130).

Types of samples	Number	Positive for *Acinetobacter* spp.
Endotracheal aspirate	68	37 (54.41%)
Wound swab	30	5 (16.6%)
Catheterized urine	27	5 (18.51%)
Blood	5	2 (40%)
Total (130)		49 (37.69%)

Table 4: Distribution of Acinetobacter baumannii among *Acinetobacter* spp. isolates (n=49).

Acinetobacter baumannii	39 (79.59%)
Other than *A. baumannii*	10 (20.41%)

the amplified product of 353 bp regions and is indicated by arrow. Lane 6 shows 100 bp ladder (Figure 1). PCR was done to detect the *TEM* gene for *Acinetobacter baumannii*, Lane 8, 9, 10, 11 are showing bands of the amplified product of 445 bp regions and is indicated by arrow. Lane 7 shows the 100 bp ladder (Figure 2). PCR was done to detect the *CTX-M* gene for *Acinetobacter baumannii*. Lane 2, 7, 9, 10, 11, 12, and 18 are showing bands of the amplified product of 593 bp regions and is indicated by arrow. Lane 8 and 17shows the 100 bp ladder (Figure 3).

Discussion

Acinetobacter baumannii is a common opportunistic pathogen that causes major nosocomial infection in hospitals. This study was conducted on critically ill patients of intensive care unit, Surgery and Burn unit and sample size was 380. Among them, 130 (34.21%) yielded growth correlates with the findings from BIRDEM hospital [16]. In India, reported 38.41% and 23.15% respectively [17,18]. Rate of culture positivity and isolation rate of organisms from various samples vary by hospitals and country.

In the present study, *Acinetobacter baumannii* was the most frequently isolated pathogen, 49 (37.69%) out of 130 organisms, which correlates with the finding of Bangladesh [19]. Study from Bangladesh reported 7.24% and 34% [5,20]. In India, reported 42.02%

Table 5: Antimicrobial susceptibility pattern of the *Acinetobacter baumannii* by disc diffusion method (n= 39).

Antimicrobial agent	(Sensitive %)	Intermediate (%)	Resistant (%)
Cephalosporin's			
Ceftazidime	7 (17.95)	2 (5.13)	30 (76.92)
Ceftriaxone	7 (17.95)	1 (11.11)	31 (79.48)
Cefepime	11 (28.21)	0 (0)	28 (71.79)
Aminoglycosides			
Amikacin	15 (38.46)	0 (0)	24 (61.54)
Gentamicin	14 (35.89)	0 (0)	25 (64.11)
Fluoroquinolones			
Ciprofloxacin	11 (28.21)	0 (0)	28 (71.79)
Levofloxacin	14 (35.89)	0 (0)	25 (64.11)
Tetracycline's			
Doxycycline	11 (28.21)	0 (0)	28 (71.79)
Folate antagonist			
Cotrimoxazole	9 (23.07)	0 (0)	30 (76.92)
β-lactam combination			
Piperacillin-Tazobactam	17 (43.59)	0 (0)	22 (56.41)
Carbapenems			
Imepenem	22 (56.41)	0 (0)	17 (43.59)
Meropenem	14 (35.89)	0 (0)	25 (64.11)
Lipopeptides			
Colistin	34 (87.18)	0 (0)	5 (12.82)
Glycyclines			
Tigecycline	39 (100)	0 (0)	0 (0)

Table 6: Frequency of studied genes in *Acinetobacter baumannii* isolates (n=39).

Name of studied genes	No. percentage (%)
*ESBL*s genes	
TEM	20(51.28)
CTX-M	16 (41.02)
SHV	0(0)

[18]. The frequencies of *Acinetobacter* from clinical samples vary by hospital, patient population, exposure to antibiotics, types of patient and changes over time.

Most of the Acinetobacter 37 (54.4%) was obtained from endotracheal aspirates in the present study which is similar to the findings of from Bangladesh [16]. Another study from India, reported 54.54%, 63.15% and 53.39% respectively [17,18,21]. From Italy [22] reported 72.2%. Higher isolation rate from endotracheal aspirates probably due to the fact that most patients either had prior respiratory problems or were in ventilators or because of low immunity or severe illness gave the best of opportunity of *Acinetobacter* commensal of upper respiratory tract to become a pathogen or from contaminated hands and objects of the health care workers.

OXA-51 like gene is a chromosomal encoded gene and intrinsic to *Acinetobacter baumannii*. Out of 49 isolated strains, 39 (79.59%) strains were positive for *OXA-51* like gene. This finding was correlates with the study from South Africa6 reported 83% positive for *OXA-51* like gene while from Saudi Arabia reported 72.7% [23].
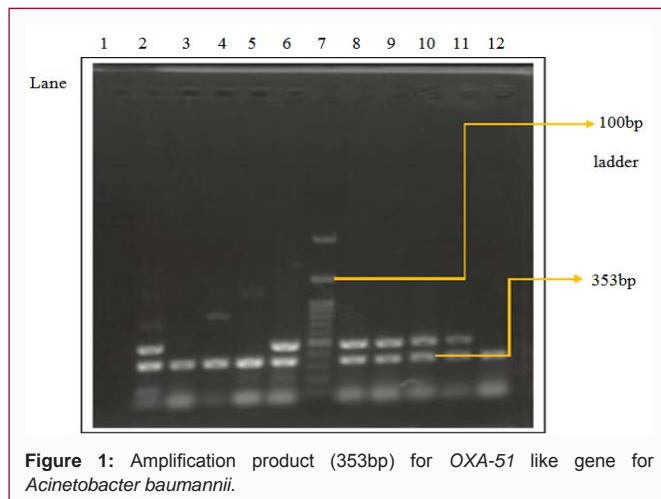

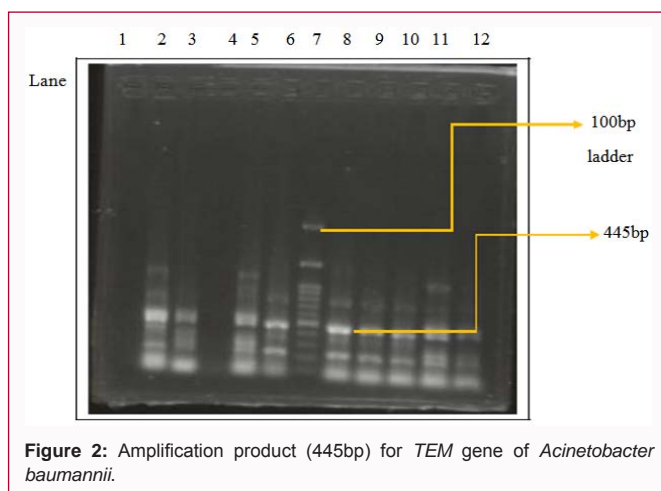**Figure 1:** Amplification product (353bp) for *OXA-51* like gene for *Acinetobacter baumannii*.

Figure 2: Amplification product (445bp) for *TEM* gene of *Acinetobacter baumannii*.
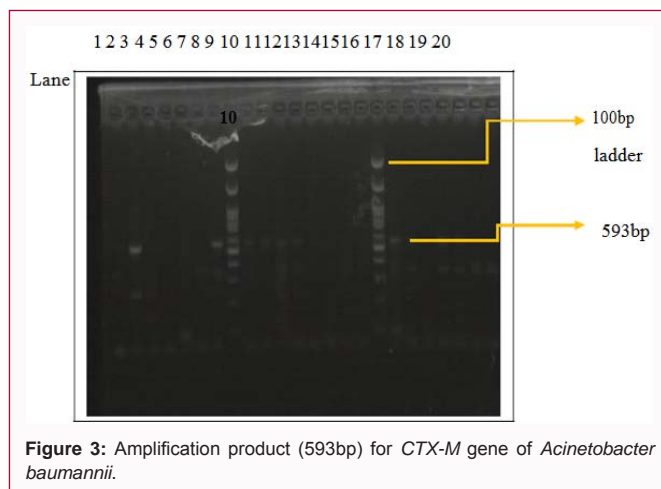

Figure 3: Amplification product (593bp) for *CTX-M* gene of *Acinetobacter baumannii*.

According to antimicrobial resistance pattern >70% strains were resistant to each Cotrimoxazole, Doxycycline, Ciprofloxacin and to 3rd generation cephalosporin, 64% to Meropenem >60% to each Gentamicin, Amikacin and Levofloxacin, 56% to Piperacillin-Tazobactam, 43% to Imepenem and 12.82% to Colistin. The antibiotic resistance observed in this study relatively similar to study conducted from Bangladesh and from Iran [24,25]. All the isolates were sensitive to Tigecycline. The difference in resistance of *A. baumannii* is probably due to diversity in clinical samples, time of

doing study and approaches of treatment in each geographical area. The high sensitivity for Tigecycline and Colistin may be so because these drugs are reserved as second-line of antibiotic. Also, these two drugs were not prescribed in ICU and others unit of Mymensingh Medical College Hospital.

ESBLs are mainly plasmid mediated and most are parts of TEM and SHV families [7,26]. In this study, 51.28% (20/39) strains were positive for TEM gene. TEM was found the most common gene in the present study. From India [27] TEM gene as the most frequent gene in 51.5% of the strains. From Iran [28] reported 52.1% of isolates carried TEM gene. In this study, 41.02% (16/39) strains were positive for CTX-M gene. From India [27] reported 30.3% strains were positive for CTX-M. Another study conducted from Iraq [29] reported 45% for CTX-M gene. In the present study no strains were positive for SHV gene. From Iran [30] reported no strains were positive for SHV gene. Therefore, the results of different studies can vary due to different region in which the studies were performed, varied prevalence of pathogenic strains carrying resistance genes especially in hospitals, abusing/overusing of antimicrobial drugs by patients, and study design in terms of methodology and sample size.

In conclusion, resistance against 3rd generation cephalosporin is high which corresponds to the genotypic ESBLs production. The epidemiologic diversity of ESBLs encoding genes in A. baumannii may suggest that new ESBLs strains are constantly emerging. Forthcoming studies emphasis on study of other ESBL encoded genes. This study revealed both the need for more caution in antibiotic consumption and the alarming rate of resistance.

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