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**Original Article**

# Detection of SHV Gene from Extended-Spectrum Beta-Lactamases (ESBLs) Producing Isolates in a Tertiary Care Hospital

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**Abstract**
**Most ESBL Extended-Spectrum beta-lactamases (ESBLs) producing isolates carry TEM and SHV type beta-lactamases. Among these two types beta-lactamases SHV type was reported nosocomial infection outbreak in many countries in the world. The goal of this study was to see the rate of ESBLs production and also to detect SHV gene in those isolates recovered from a tertiary care hospital. A total of 354 samples were collected from Sir Salimullah Medical College and Mitford Hospital and from Burn Unit of Dhaka Medical College Hospital from January 2011 to December 2011. To detect ESBLs, Gram-negative organisms were subjected to Double Disc Synergy Test and SHV gene was detected from those ESBLs producing microorganisms by PCR. In this study, out of 354 different clinical samples 186 (89%) were Gram-negative organisms. Among of these Gram-negative organisms 77 (41.40%) were ESBL producing isolates detected by Double Disc Synergy Test. Out of these 77 isolates, 8 isolates (10.38%) carried *bla* SHV gene. Among those ESBL producing 26.26% *Klebsiella spp.* (4 out of 15 isolates) had SHV gene. Our findings showed that the majority of the ESBLs positive clinical isolates were *Klebsiella spp.* and high frequency of *SHV* genes were found in *Klebsiella spp.***

**Keywords: Extended spectrum Beta-lactamase (ESBL), Double Disc Synergy Test, Polymerase chain reaction.**

## Introduction:

Extended-spectrum beta-lactamases (ESBLs) are the rapidly evolving group of beta-lactamase enzymes produced by the Gram negative bacteria, which have the ability to hydrolyse all cephalosporins and aztreonam but are inhibited by clavulanic acid[1]. Incidence of ESBL producing strains are steadily increasing over the past years resulting in limitation of therapeutic options[2]. Early detection of multi resistant bacteria is important in defining therapies and for the isolation of patients, which is necessary to prevent the spread of these pathogens and also to prevent hospital-acquired (nosocomial) infections and outbreaks in the community[3,4]. Most ESBLs found in *Esch. coli* and *Klebsiella pneumoniae carry* the TEM and SHV type of ß-lactamases[5]. These enzymes are generally located on large, transferable plasmids responsible for dissemination[6]. Since 1984, multi resistant *Klebsiella pneumoniae* has been increasingly recognized as a cause of nosocomial infections[7].

The SENTRY Antimicrobial Surveillance Program showed that ESBLs producing *K. pneumoniae* isolates were more prevalent in Latin America (45.5%), followed by the Western Pacific region (24.6%), Europe (22.6%), the United States (7.6%) and Canada (4.9%). In Asia the prevalence of ESBL-producing *K. pneumoniae* and *E. coli* vary from 5% in Japan to 20-50% in other countries. In Europe, the prevalence of these organisms varies from country to country (3% in Sweden to 34% in Portugal)[8,9,10]. The colonization rate for *Klebsiella pneumoniae* is low in healthy individuals in the general population. But it is increased in hospitalized patients especially with long care facilities, health care manipulations eg. use of catheters[11]. The SHV-1 ß-lactamase is most commonly found in *Klebsiella spp*. ESBLs in this family have amino acid changes most commonly at position 238 or 238 and 240. They are the predominant ESBL type in Europe, United States and are found world wide[12]. Among the SHV-type of ß-lactamases, SHV-5 is found to be responsible for outbreaks of nosocomial infection in several countries[13].

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SHV-5 is transferred from *Klebsiella pneumoniae* to *Escherichia coli* as well as to other members of *Enterobacteriaceae* family and poses a serious problem in the control of antibiotic resistance[14]. *Klebsiella pneumoniae* is an opportunistic pathogen that causes a significant proportion of community and hospital acquired infections including urinary tract, pneumonia, septicemia and soft tissue infections[15]. The prevalence of chromosomally encoded SHV ß-lactamase was reported worldwide during 2000 and 2001[16,17]. SHV-type ESBLs currently predominate in surveys of resistant clinical isolates in Europe and America[18].

Though presence of ESBLs have been reported from Bangladesh there is paucity of data regarding their molecular types. So the main aims of this study were to assess rate of ESBL producing isolates amongst the patients admitted in a tertiary care hospital in Bangladesh, alongside occurrence of SHV gene in those ESBL producing isolates by polymerase chain reaction.

**Materials and Methods**

This Cross sectional study was carried out in the department of Microbiology, Sir Salimullah Medical College, Dhaka for a period of one year from January 2011 to December 2011. A total 354 samples were collected from in patient and out patient departments of Sir Salimullah Medical College & Mitford Hospital and Burn Unit, Dhaka Medical College Hospital. Hence, 3 categories of patients were included in this study, clinically diagnosed UTI patients, infected burn patients, patients with infected wound (surgical wound). All ESBL producing isolates detected by Double Disc Synergy Test. Klebsiella pneumoniae ATCC 700603 and Escherichia coli ATCC 25922 were used as positive and negative control respectively for ESBLs detection[19].

**Culture**

Organisms (urine samples, surgical wound swab, burn wound swab, pus) were isolated by inoculating onto Blood agar and MacConkey's agar media and were identified by standard biochemical tests[20].

**Double Disc Synergy Test**

Isolated Gram-negative bacteria were subjected to DDST. ESBLs production was considered positive when the inhibition zone around the test antibiotic disc was increased towards the augmentin disc (20µg amoxicillin+10µg clavulanic acid) which was placed in the centre of the plate and 20 mm apart from other discs[21].

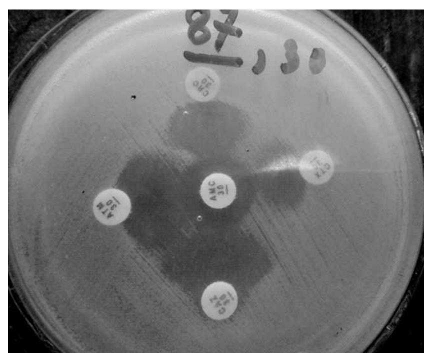


Figure-I: Positive ESBL isolate detected by DDST.

**Identification of SHV gene among the ESBL producing isolates by PCR:**

**Broth culture:** Stored organisms were subculture into Mueller-Hinton agar media from Nutrient agar slant. Bacterial colonies (5 colonies) were taken from Mueller-Hinton media using wire loop and inoculated into test tube containing Tryptic soy broth (3ml). Test tube was incubated at 37°C for 24 hours and was centrifuged at 4000 rpm for 10 minutes. Supernatant was removed and the pellets were then transferred into an eppendorf tube for DNA extraction[22].

**DNA extraction:**

A standard protocol was used for extraction of DNA. For the rapid lysis procedure, pellets were mixed with 300 µl of distilled water. Bacterial suspension was heated at 95°C for 10 minutes and then quickly placed on ice. This suspension was then micro centrifuged at 14000 rpm for 10 minutes. Supernatant was used as DNA extract. The supernatant was then taken in another eppendorf tube and stored at -20°c. The extracted DNA was used for amplification of DNA by PCR as template DNA.

**PCR Primers:**

**Table I: Characteristics of the PCR primers of SHV gene[19]:**

| Primers | Sequence 5′-3′ | Primer Size (bp) | Size of the PCR products in bp |
|---|---|---|---|
| SHV-F | GGG TTA TTC TTA TTT GTC GC | 20 | 30 |
| SHV-R | TTA GCG TTG CCA GTG CTC | 18 | |

SHV-F = Sulfhydril variable Forward
SHV-R = Sulfhydril variable Reverse

**Steps involved in PCR protocol [19]**

The reaction was performed in a thermocycler using the following steps :

1. Initial denaturation of template DNA: Initial denaturation at 94°C for 5 minutes.

2. Final denaturation of template DNA : 35 cycles at 94°C for 1 minute.
3. Annealing of primer : 52°C for 1 min.
4. Extension of primer : 72°C for 1 min.
5. A final extension for 10 minutes at 72°C was done to complete the synthesis of all amplicon.

The whole process of amplification took about 2.5 hours to complete PCR reaction.

**Detection and visualization of PCR products:**

The amplified PCR products were detected by 0.8% agarose (Invitrogen) gel electrophoresis, and then staining was done with ethidium bromide. The electrophoretic band was visualized in a gel documentation system by using UV transilluminator.

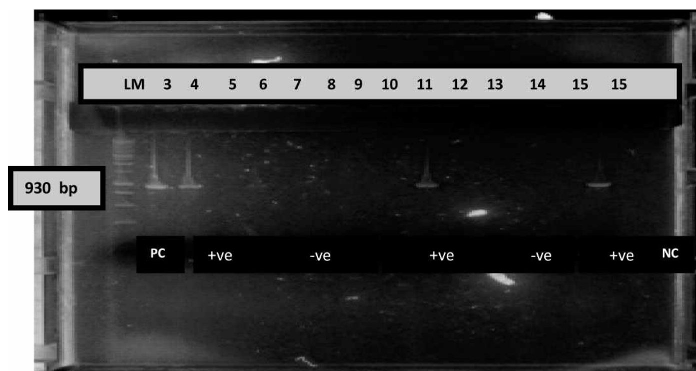


Figure (II): Agarose Gel showing fragment of *bla* SHV at 930 bp.
PC = Positive control
NC = Negative control
LM = Ladder
Lane 2 Ladder
Lane 3 Positive control (*Klebsiella pneumoniae* ATCC 700603)
Lane 4, 10, 14 are positive samples
Lane 15 Negative control (*Escherichia coli* ATCC 25922)

**Results**

Table-II shows rate of isolates of Gram-negative bacteria among total number of isolates from SSMC & MH and DMCH. Among 354 samples 209 isolates were detected. Out of them 186 isolates (89%) of Gram-negative bacteria had been identified from both hospitals.

Table-III shows among 186 isolates 77(41.40%) isolates were phenotypically confirmed by DDST. Among them highest percentage of ESBL producing isolates were found in *Klebsiella spp.* Out of 20 *Klebsiella spp.* 15(75%) isolates were phenotypically confirmed as ESBLs followed by *Proteus spp.* (45.45%), *Esch. coli* (41.66%) and *Pseudomonas spp.* (21.21%).

Table-IV denotes presence of SHV gene among phenotypically confirmed ESBL producing isolates. Among 77 phenotypically confirmed ESBL producing isolates SHV genes were detected in only 8 isolates. Out of 50 ESBL producing *Esch. coli,* 4 isolates were (8%) carrying SHV gene and among 15 ESBL producing *Klebsiella spp.*, 4 isolates (26.67%) were having SHV gene. No isolates of *Proteus spp.* and *Pseudomonas spp.* carried SHV gene.

**Table II: Rate of isolates of Gram-negative bacteria among total number of isolates from SSMC & MH and DMCH**

| Name of hospitals | Total No. of tested samples | Total No. of isolates | Number of Gram-negative bacteria |
|---|---|---|---|
| SSMC & MH | 300 | 157(52.33) | 138(87.90) |
| DMCH | 54 | 52(96.30) | 48(92.31) |
| Grand Total | 354 | 209(59.04) | 186(89) |

Note: Figures in parentheses represent percentage.

**Table III: Distribution of ESBL producers detected by DDST among all Gram-negative organisms**

| Name of isolates | DDST positive |
|---|---|
| *Esch. coli* (n = 120) | 50(41.67) |
| *Pseudomonas spp.* (n = 33) | 7(21.21) |
| *Klebsiella spp.* (n = 20) | 15(75) |
| *Proteus spp.* (n = 11) | 5(45.45) |
| *Acinetobacter spp.* (n = 2) | 0(0) |
| **Total (n = 186)** | **77(41.40)** |

Note: Figures in parentheses represent percentage.

**Table IV: Presence of SHV gene among phenotypically confirmed ESBL producing isolates**

| Name of isolates | Double Disc Synergy Test positive | SHV gene | |
|---|---|---|---|
| | | Present | Absent |
| *Esch. coli* | 50 | 4 (8) | 46(92) |
| *Klebsiella spp.* | 15 | 4(26.67) | 11(73.33) |
| *Proteus spp.* | 5 | 0(0) | 5(100) |
| *Pseudomonas spp.* | 7 | 0(0) | 7(100) |
| **Total** | **77** | **8(10.39)** | **69(89.61)** |

Note: Figures in parentheses represent percentage.

**Discussion:**

Infectious diseases and their treatment are important problems in mankind's life and daily increase in bacterial resistance has raised patients expenses in recent years. ESBL production rate by *Enterobacteriaceae* has increased noticeably in two recent decades. Most of the hospitalized patients have immune deficiency and underlying disease and *K. pneumoniae* as an opportunistic pathogen is one of the

most important causes of nosocomial infections especially in surgical wards and intensive care units[23]. There are different reports from all over the world in the case of prevalence of ESBLs bacteria. In the present study, out of 354 different samples 209 (59.04%) organisms were isolated. Among them 186 (89%) were Gram-negative organisms (Table-II). Among the 186 Gram-negative bacteria, phenotypically confirmed ESBLs were 77 (41.40%) (Table-III). Majority of ESBL producers were observed among *Klebsiella spp.* (75%) followed by *Proteus spp.* (45.45%), *Esch. coli* (41.67%), and *Pseudomonas spp.* (21.21%). It is important to note that the percentage of ESBL producers has increased in this hospital from 25.23% to 41.40% over the last 4 years possibly due to indiscriminate and inappropriate use of 3rd generation of cephalosporin[24,25]. The prevalence of ESBLs has been reported to be 20% in some studies in southern east of Asia, in some regions it has been more than 60% [26]. A study in the year 2004 has shown the prevalence of ESBLs in Europe to be 18.4% which is 40% in Netherland and 3% in Sweden[27]. In the present study, ESBL producing *Klebsiella spp.* was more. The reason of this might be collection of samples from hospitalized patients and post-operative patients. Overcrowding, under stuffing or nursing workload might be the reasons for higher yielding of ESBL producing *Klebsiella spp.* in hospital environment. In this study phenotypically confirmed 77 ESBL producing isolates by DDST were also subjected to Polymerase chain reaction to see the presence of SHV gene. Among 77 ESBL producing isolates 8 isolates (10.38%) carried *bla* SHV gene (Table-IV). Among 50 isolates of *Esch. coli,* 4 isolates (8%) carried SHV gene and out of 15 isolates of *Klebsiella spp.,* 4 isolates (26.26%) had SHV gene. SHV gene was not identified among *Proteus spp.* and *Pseudomonas spp.* (Table-IV). In a study at Indian hospital found SHV gene in 8.4% *Klebsiella spp*[28]. In contrast to the findings of that the percentage of SHV gene carrying ESBL isolates was more in the present study. In another study reported that 20.3% isolates of *Klebsiella spp.* were containing SHV genes[23]. A study revealed that 50% of *Klebsiella spp.* had SHV-1[23]. Now a days SHV-1 has been the most prevalent gene in most of the regions[29].

To conclude, phenotypic methods are only screening methods for detection of ESBLs in a routine laboratory but genotypic methods help us to confirm the genes responsible for ESBLs production. Genotypic method provided an efficient, rapid differentiation of ESBLs. The correct identification of the genes involved in ESBL mediated resistance is necessary for the surveillance and epidemiological studies of their transmission in hospitals. Appropriate antibiotic policy and infection control measures in hospital settings are crucial to overcome the problems associated with infections by ESBL producing strains.

**Limitation:**

Although utmost sincerity and dedication was investigated to carry out the study it could not go beyond limitations. As PCR reagents are expensive so all Gram-negative bacteria were not done for PCR. Samples were subjected to PCR which were only detected by DDST. Other types of genes were also not be done due to same reasons.

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