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ORIGINAL ARTICLE

Detection of Extended spectrum beta lactamase in *Escherichia coli* isolated from various clinical samples in a tertiary care hospital in Assam, India

Shila Kumari Singh*, Vekutolu Resuh, Lucky Ahmed, Elemi Debbarma
Faculty of Paramedical Science, Assam downtown University, Guwahati, Assam, India.
***Correspondence: Email: shilasingh82@gmail.com**

ABSTRACT

Extended spectrum beta lactamase is an enzyme that is common among the members of enterobactericae. UTI caused by ESBL containing strains of Escherichia coli are widely prevalent. Failing to detect the ESBL producing ability of the organism can lead to failure of treatment, particularly with penicillin and cephalosporin. The aim of the study is to detect the prevalence of ESBL production in E.coli causing urinary tract infections. This study also detects the performance of double disc synergy test to detect ESBL in Escherichia coli. Total 80 strains of Escherichia coli are isolated from cases of Urinary tract infection. Identification of the organism was done by microscopy and biochemical tests. Antibiotic sensitivity test is done to detect the pattern of resistance among the organisms. After the sensitivity test the strains of Escherichia coli are tested by double disk diffusion method for the ability to produce ESBL. Out of the total 80 strains of E.coli, 28 ESBL producers were detected and 52 non- producers. As compared to the non-producers, ESBL producers were found to be multi drug resistant. In our study, we found 35% prevalence of ESBL producing Escherichia coli in UTI cases. The prevalence of ESBL in Uropathogens has increased in India and worldwide over the years. We used DDST method for the detection of ESBL which is a convenient, reliable and cost-effective phenotypic method that can be used by laboratories with limited resources. Detection of ESBL can help in the controlling the failure of treatment. This can improve the overall outcome of healthcare system.

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INTRODUCTION

Escherichia coli is considered as one of the most common pathogen causing nosocomial infection in humans, such as urinary tract infection (UTI), blood-stream infection (BSI), and gastrointestinal infection.[1] Severe infections at non-intestinal sites like urinary tract, bloodstream, cerebrospinal fluid are frequently seen in Extra-intestinal pathogenic *Escherichia coli* (ExPEC) that are usually common both in hospital and community settings. The potential of ExPEC isolates to carry specific virulence genes adds to a success in colonizing tissues and survive out of the intestinal habitat which cause them to progress in extra-intestinal disease. [2] Extended spectrum beta-lactamases (ESBLs) is an enzyme that is produced by certain strain of bacteria which breaks the beta-lactam ring of the cephalosporin. ESBLs are consequently effective against the beta-lactam antibiotics like Ceftazidime, ceftriaxone and cefotaxime. [3] ESBL producing bacteria are responsible for high morbidity, mortality, and health care costs. [4] This enzyme can lead to multi-drug resistance (MDR), which will reduce the available options of antimicrobial agents for treating ESBL infections. [1]. *Escherichia coli* producing ESBL has emerged as a threat to human being. Its ability to infect healthy individuals in the community and immunocompromised patients in hospitals is a point of concern. Worldwide cases of ESBL producing *Escherichia coli* infecting healthy individuals have increased in the community settings. [5, 6, 7] The enzyme ESBL plays a major role in the antibiotic resistance and leads to serious infections in hospitalized patients who are immunocompromised. The CTX-M ESBL producers or Cefotaximase producing ESBL show co-resistant to Fluroquinolones in addition

to Cefotaxime.[8] Studies have shown that developing countries have an increased prevalence of ESBL producing organisms when compared to countries with better resources and medical facilities. The common reason for this is found to be limited resources leading to crowding of hospitals and unhygienic practices and indiscriminate use of antibiotics. [9]. Currently, the detection of ESBL is difficult for the routine clinical microbiology laboratories with limited resources. The Comite de I'Antibiogramme de la Societe Francaise de Microbiologie (CA-FSM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) suggest screening of ESBL producing isolates based on their resistance to the extended spectrum cephalosporin in the routine antibiotics sensitivity testing and then confirmation by an additional method like Double disc synergy test (DDST). This method was designed to differentiate between resistance towards third generation cephalosporin and ESBL producers.[8,9] As it is not yet confirmed that which confirmatory phenotypic test is best, other methods of detection like inhibitory potential disk diffusion or E strip confirmatory test can be used too.[10] . In this study, *Escherichia coli* isolated from various clinical samples were identified and tested for their ability to produce ESBL. In addition to the antibiotic sensitivity testing we included the double disk synergy method to detect the ability of the organism to produce ESBL. This method is cost effective and sensitive which makes it a suitable and sustainable method for laboratories with limited resources.

MATERIAL AND METHODS

This study was conducted in Assam downtown University and samples were collected from downtown hospital for a period of 4 months from April 2022 to July 2022.

Collection of Samples:

This study included *Escherichia coli* that are isolated from various clinical samples like Urine, pus, stool, vaginal swab, body fluids etc during the study period. Total 80 strains of non duplicate *Escherichia coli* were included in this study.

Identification by biochemical tests:

All the gram negative bacilli are identified first by microscopy and then by biochemical tests like catalase, coagulase, oxidase test, motility test, Indole production test, MR-VP test etc.

All gram negative motile bacteria which are identified as *Escherichia coli* based on the findings of all the biochemical tests are included in this study. Total 80 isolates of *Escherichia coli* we isolated and identified.

Antibiotic sensitivity testing:

All the isolates of *Escherichia coli* were tested for their susceptibility to antibiotics by Vitek 2 compact using GN cassette card. The turbidity of the bacterial suspension was compared to 0.5 as per McFarland standard by DensiCHEK Plus. The bacterial suspension was inoculated into the Vitek-2 compact along with GN cassettes AST card. Interpretation of the AST results was performed according to the Vitek-2 compact system. The final interpretation and analysis was completed by using the Clinical Laboratory Standards Institute (CLSI) criteria. The antibiotics used in this study were Ticarcillin, Amoxicillin/Clavulanic acid, Piperacillin, Cefepime, Ceftazidime, Cefalotin, Cefoxitin, Cefixime, Ceftriaxone, Aztreonam, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Trimethoprim /sulfamethoxazole, Minocycline, Piperacillin/tazobactam, Imipenem, Meropenem, Fosfomycin, Nalidixic acid, Nitrofurantoin and Ertapenem. [11]. Additionally, ATCC Escherichia coli, 25922 was tested along with the test strains as a control for the antibiotic sensitivity test.

ESBL detection by DDST (Double disc synergy test) method:

After performing the antibiotic sensitivity test, strains showing resistance to any of the three-indicator cephalosporin were tested for ESBL production by the DDST method. We followed the procedure given by Harwalkar et al., in 2013. [10] In this test one antibiotic discs of third generation cephalosporin [Ceftazidime (30 µg) or cefotaxime (30 µg)] and amoxicillin/clavulanic acid disc (amoxicillin 20 µg + clavulanic acid 10 µg) are used for ESBL detection. The test organism inoculum is matched with 0.5 McFarland Standard and inoculated on Muller hinton agar by lawn culture method and allowed to dry for about 5 minutes. Antibiotic disks of amoxicillin-clavulanic acid (20/10 µg) is placed at the centre of a Mueller-Hinton agar plate after that place either cefotaxime (30 µg) or Ceftazidime (30 µg) disc at a distance of 15mm (centre to center) from the centre disc and incubate at 37°C for 18 to 24 hours. ESBL production is interpreted as positive when the zone of inhibition of the cephalosporin (Cefotaxime or Ceftazidime) is enhanced at the side facing the amoxicillin-clavulanic acid (AMC) disks. (figure 1). ESBL producing ATCC Klebsiella pneumonia 700603 and non ESBL producing ATCC *Escherichia coli* 25922 are used as positive and negative control respectively.

Figure 1: **Double disk diffusion test detecting ESBL.**

RESULT

In our study we used the double disc diffusion methods to identify ESBL production in *Escherichia coli* isolated from Urine samples. Out of the 80 strains 28 (35%) showed ESBL production and 52 (65%) were non producers of ESBL.

The antibiotic sensitivity pattern of ESBL producers and non producers are shown below in Table1.

Table 1: Resistance pattern of ESBL producers and Non-Producers

Antibiotics	**Resistance in ESBL non producers (n=52)**	**Resistance in ESBL producers (n=28)**
Ampicillin (AMP)	33(63%)	20 (71%)
Amoxicillin (AMX)	13(25%)	5 (17%)
Ticarcillin (TIC)	31(59.6%)	18 (64%)
Piperacillin (PIP)	8(15%)	4 (14%)
Cefalotin (CEF)	26(50%)	15 (53%)
Cefoxitin (CX)	12(23%)	4 (14%)
Cefixime (CFM)	20(38%)	16 (57%)
Ceftazidime (CAZ)	11(21%)	14 (50%)
Ceftriaxone (CRO)	22(42%)	18 (64%)
Ertapenem (ETP)	5(9.6%)	5 (17%)
Amikacin (AMK)	3(5.7%)	4 (14%)
Gentamicin (GEN)	3(5.7%)	5 (17%)
Nalidixic Acid(NAL)	40(76.9%)	19 (67%)
Ciprofloxacin (CIP)	24(46.1%)	18 (64%)
Norfloxacin (NOR)	15(28.8%)	13 (46%)
Ofloxacin (OFX)	11(21%)	17 (60%)
Trimethoprim (TMP)	34(65%)	14 (50%)
Cefoperazone (CEP)	5(9.6%)	5 (18%)
Cefuroxime (CXM)	0(0)	4 (14%)

Table 2: Distribution of ESBL producers based on Age, Sex and OPD/IPD

Age	**Children (0-17years) (n=11, 39%)**	**Adult (18-45years) (n=13, 46%)**	**Older adults (46-70 years) (n=4, 14%)**
Male	8 (28%)	2 (7%)	2 (7%)
Female	3 (10)%	11 (39%)	2 (7%)
OPD	9 (32)%	13 (46%)	3 (10%)
IPD	2 (7%)	-	1 (3%)

The ESBL producers were more prevalent in age groups between 18-45 years. Female showed more cases 16(57%) as compare to male 12 (43%). Most of the ESBL producers 25(89%) were isolated from OPD patients **(Table 2).** We also compared the prevalence in pregnant and non-pregnant women and found that out of 16 ESBL producers were isolated from female patients, 7 of them were pregnant and 9 non pregnant.

DISCUSSION

ESBL is reported as an enzyme that has increased overtime and currently stands as a major concern for healthcare system with reference to hospital as well as community. [12] As reported, urinary tract infections(UTI) are the most common type of hospital acquired infections and *Escherichia coli* is reported to be the most prevalent organism causing UTI.[13,14] In our study, we included 80 cases of urinary tract infection where *Escherichia coli* was isolated as the pathogen. Out of the 80 isolates we detected ESBL in 28 (35%) and rest 52(65%) were non ESBL producers by double disk synergy test. Reports published in last decade have shown variable values but definitely a rise in the cases of ESBL is seen. [15-21] In a retrospective study conducted by Vachvanichsanong, P. et al in 2021, *E.coli* and Klebsiella species were the common among the children with UTI and they found recurrent UTI to be a significant risk factor for ESBL associated UTI, in our study children from age group constituted 39% of the total ESBL producers. [22] Lindblom, A., et al in 2022 also published the similar findings where they found patients with ESBL *E.coli* associated UTI have recurrent UTI with ESBL E>coli as the causative agent.[23]. Based on earlier reports ESBL associated UTI is equally affecting hospital and community settings. [24] Most of our ESBL positive strains were from OPD patients from community settings. Earlier reports also suggest prevalence of UTI in community as compared to hospitals.[25,26] Recent studies have revealed the gradual increase in multidrug resistant ESBL associated UTI in both hospital and community.[26] . The Laboratory diagnosis of ESBL is very important to avoid the failure of treatment; many laboratories are unaware of its importance. This has led to the spread and outbreaks of MDR ESBL in community and hospitals.[27] In a country like India, where resources are limited, a reliable and cost effective method is very useful. Many studies have reported double disk synergy test as a reliable and cost effective test to detect ESBL. Among the phenotypic methods available for detection of ESBL, DDST is considered to be most reliable one. DDST method can work even in the absence of combination drugs and it can be carried out during routine antibiotic sensitivity test. These attributes of DDST test makes it very useful in diagnostic laboratories with limited resources.[28]

CONCLUSION

As the cases of ESBL have become very common and it is affecting both the community as well as the hospital settings, it is necessary to detect ESBL without fail. In this study DDST was used and found reliable. Molecular diagnosis of ESBL by detecting the associated genes is considered as the gold standard but DDST can also be used in laboratories lacking the setup for genotypic studies.

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