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Molecular Characterization Of Extended Spectrum B-Lactamase (ESBL) Producing E.coli Isolates With Special Reference To Blatem And Blashv Gene From Patients Of Urinary Tract.

Dr. Raees Ahmed[1], Dr. Sheetal Agarwal[2], Dr. Vanshika Sharma[3], Dr. Nashra Afaq[4], Dr. Mukesh Kumar[5], Dr. Deepika Shukla[*6]

[1]Assistant Professor, Department of Microbiology, Sudha Medical College and Hospital, Jagpura, Kota, Rajasthan, India.

[2]Senior Resident, Department of Microbiology, King George Medical University, Lucknow, Uttar Pradesh, India.

[3]Junior Resident, Department of Microbiology, Government Medical College, Jammu, India.

[4]Research Associate, Department of Microbiology,and Central Research Lab, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India.

[5]Junior Resident, Department of Microbiology, King George Medical University, Lucknow, Uttar Pradesh, India.

[*]Associate Professor and Head, Department of Microbiology, Maharana Pratap Dental College and Hospital Kothi, Uttar Pradesh, India.

Corresponding Author: Dr. Deepika Shukla*
Email ID: deepikashukla60@gmail.com

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ABSTRACT

Introduction: ESBL allied infections are stairing up worldwide and has emerged to be a grave problem to public health. β-Lactams are among the most widely prescribed antibiotics in human medicine. However, because of their massive and usually inappropriate use, resistance to these drugs has increased markedly, especially due to extended-spectrum β-lactamase (ESBL) production.

Aim and Objective: To study the molecular characterization of extended spectrum β-lactamase (ESBL) producing *E.coli* isolates with special reference to *blaTEM* and *blaSHV* gene from patients of urinary tract.

Material and Methods: This was a Cross sectional study carried out in the department of Microbiology for a period of 12 months i.e, October 2023 to October 2024. A total of 366 *E. coli* isolates of all the Uropathogenic*E. coli* strain isolated from urine samples collected from hospitalized and consultation patients were included in the study. The Antimicrobial susceptibility testing was performed according to the CLSI guidelines 2024. The DNA extraction was done using the Qiagen DNA extraction kit and the gene blaTEM and blaSHV was detected using the PCR.

Results: Out of the 900 isolates there were 366 (40.6%) which showed the isolates of *E. coli*. In which 82 (22.5%) were phenotypically identified as ESBL producers and 284 (77.6%) were Non-ESBL. Out of 366 patients who were included in this study 110 (30%) were Males and 256 (69.9%) were Females patients. The overall susceptibility of ESBL isolates to

various antibiotics was as Ampicillin(17.20%), Ampicillin/Sulbactam (28.5%), Gentamycin (65.7%), Cefoxitin (51.0%), Amikacin (80%), Ciprofloxacin (48%), Meropenem (97.2%), Ceftazidime(0%), Ceftazidime/ clavunalate(100%), Piperacillin/tazobactam (85.7%), Ceftriaxone(0%), Nitrofurantoin(100%), Tigecycillin(97.2%) and fosfomycin(97.2%). In the current study out of the total 82 isolates the molecular method confirms that there were 40 (48.7%) observed positive for blaTEM gene and 11 (13.4%) observed positive for blaSHV gene.

Conclusion: The significant spread of *E. coli* that produces ESBL has resulted in fewer treatment options and higher medical expenses. In order to support appropriate antibiotic therapy, as well as efficient infection control and clinical care management, it is imperative that trends for regional epidemiological data on antimicrobial resistance be updated.
Keywords: ESBL, Urinary Tract Infection, MDR, β-Lactams , CLSI

Introduction

Escherichia coli, a member of the *Enterobacteriaceae* family, has been reported to be one of the most predominant organisms causing urinary tract infections (UTIs) which are very common reasons for consultation and antibiotic prescription in current practice [1]. Urinary tract infections (UTIs) affect people all over the world, especially in India, and are a major cause of community and healthcare-associated illnesses [2]. Urinary tract infections affect between thirty and fifty percent of people, and they can cause infections. This high frequency is linked to a number of risk factors, such as past hospitalisation, immunosuppressive medication use, diabetes, urinary tract blockages, surgical manipulation, catheterisation, and other co-morbidities. Different risk factors and antibiotic resistance profiles apply depending on whether the infection was acquired in the community or in a hospital [3,4]. Even though most UTIs are not severe, hospitalised individuals with UTIs have a higher morbidity rate and are more likely to acquire sepsis.

Escherichia coli is the primary cause of UTIs, accounting for 70–80% of cases [5,6]. The quick increase in antibiotic resistance has made treating UTI patients more difficult. [7] Although *E. coli's* creation of the extended-spectrum β-lactamase (ESBL) enzyme is a serious issue, the sharp rise in fluoroquinolone and aminoglycoside resistance has also greatly influenced the few and difficult treatment options available to infected people [8, 9]. Numerous studies have shown that a significant portion of UTIs in hospitals and communities are caused by *E. coli*, which produces ESBLs [10,11].

However, the plasmid triggered a naturally occurring β-lactamase enzyme in other bacterial species. The misuse of β-lactam antibiotics in gram-negative bacteria led to the development of broad spectrum enzymes like TEM-1 and SHV-1 after the introduction of first and second generation cephalosporins [12]. Later, extended spectrum β-lactam antibiotics were introduced using enzymes that were detrimental to hydrolysis. The oxyamino-ceplalosporinsceftazidine and cefotaxim are frequently utilised. These more recent medications were hydrolysed as a result of the creation of novel β-lactames [13].

ESBLs have been reported in India most frequently in Enterobacteriaceae and cause difficulty in the management of infections in intensive critical care settings. The prevalence of ESBLs resistance varies greatly in geographic areas, and the trend keeps on changing over time at a rapid pace. CTX-M, TEM, SHV-type ESBL have become prevalent worldwide including in India, and enzymes are becoming increasingly expressed by pathogenic bacterial strains in hospitals and have the potential for widespread dissemination. ESBLs such as OXA-1, PER-type, GES-type, and VEB-type have also been reported from various countries. Different investigators across India have reported prevalence ranging from 7.0 to 91% by different methods to detect ESBL resistance [14].

Massive and usually inappropriate use of antibiotics for treatment of UTIs generates a selective pressure that is followed by the rapid emergence and spread of multi-drug resistant bacterial strains. Nowadays, resistance of uropathogenic *E. coli* to many antibiotic classes is a very common finding in human medicine and is usually associated

with increased medical costs, prolonged hospital stays and frequent therapeutic failure [3].

The extended-spectrum β-lactamases, which are currently found all over the world, are the main class of enzymes used in epidemiology. According to Ambler's molecular and structural classification system, ESBLs fall under class A.A. Their ability to hydrolyse broad-spectrum β-lactam antibiotics and their resistance to β-lactamase inhibitors, especially clavulanate, set them apart biochemically. [14,15]

Polymerase chain reaction (PCR)-based molecular methods are rapid, accurate, and have better sensitivities for detecting ESBL-resistant genes than conventional phenotypic methods. They help clinicians with a targeted approach for the treatment and also help in containment of outbreaks and the implementation of infection control policies.

Therefore the present study was undertaken to study the molecular characterization of extended spectrum β-lactamase (ESBL) producing *E.coli* isolates with special reference to *blaTEM* and *blaSHV* gene from patients of urinary tract.

MATERIAL AND METHODS

This was a Cross sectional study carried out in the department of Microbiology for a period of 12 months i.e, October 2023 to October 2024 at a tertiary care centre. A total of 366 *E. coli* isolates of all the Uropathogenic*E. coli* strain isolated from urine samples collected from hospitalized and consultation patients were included in the study. The Antimicrobial susceptibility testing was performed according to the CLSI guidelines 2024.

The study population included patients of all age groups and included both male and female were included

- To detect Extended spectrum beta lactamases in *E. coli* isolates from urinary tract infection by phenotypic method combination dics diffusion test.
- To study Antimicrobial susceptibility of novel beta-lactum / beta-lactamases inhibitor combination drugs (ceftazidime-avibactam).
- To detect *bla* SHV and *bla*TEM gene in these multidrugs resistance isolates by PCR.

PROCESSING IN LABORATORY

All the Urine sample were collected in a clean universal container & processed according to standard laboratory protocols. In order to confirm UTI, urine samples were also examined under a microscope, paying special attention to any pus cell presence. One microliter of urine was inoculated with a medium lacking in cysteine lactose electrolytes deficient agar (CLED; Hi-Media Laboratories, Mumbai, India). Customary biochemical tests were used to identify the bacterial culture that flourished under pure culture and in large numbers ($>10^5$ cfu/ml for midstream urine samples). Their antibiotic vulnerability was also assessed in accordance with CLSI criteria.

ANTIBIOTIC SUSCEPTIBILITY TESTS

The samples were assessed for their vulnerability by disc diffusion technique (DDT) as per the CLSI guidelines 2024. The subsequent discs of antibiotics (drug concentrations in µg) were used: like Ampicillin(10), Ampicillin/ Sulbactam (10/20), Gentamycin (30), Cefoxitin (30), Amikacin (30), Ciprofloxacin (10), Meropenem (10), Ceftazidime(30), Ceftazidime/ clavunalate(30/10), Piperacillin/ tazobactam(100/10), Ceftriaxone(30), Nitrofurantoin(30), Tigecycillin(15) and fosfomycin (20).

PHENOTYPIC METHOD FOR DETECTION OF ESBL

Production of ESBL was confirmed with disk diffusion test using 30 µg ceftazidime (CAZ) and with a combination of 30µg +10µg ceftazidime along with clavulanic acid (CAC) discs (Hi-media, Mumbai) placed at a distance of 25 mm on a Mueller-Hinton Agar plate incubated by a bacteria (standard of 0.5 McFarland turbidity) and further kept alive of night long at 37 ◦C. A ≥ 5mm increase in inhibition diameter diameter of inhibition area for the mixture disc against disc of ceftazidime establish the systhesis of ESBL. *Escherichia coli* ATCC 25922 was utilized as positive ESBL control strain throughout our study.

GENOTYPIC METHOD

MOLECULAR METHODS: For the detection of the gene blaTEM and blaSHV gene the chromosomal DNA from the clinical strains of *E.coli* was extracted. The DNA extraction was carried out using a commercial available DNA extraction kit (Qiagen DNA Extraction Kit) as indicated by the manufacturer's instructions.

The extracted DNA was run in PCR for its extension according to standard method.

Fig No.1 The DNA Extraction Reagents

DNA extraction and PCR method

- Total genomic DNA was extracted from all the ESBL positive isolates using a DNA extraction kit according to the manufacturer's instructions. Amplification and detection of the considered gene was done by the PCR method using specific primers.
- The primers were purchased from "Saha gene' and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

Fig No. 2: Primers for TEM gene

Fig No.3: Primers of SHV gene

Polymerase Chain Reaction (PCR)

- The amplification of the blaTEM and blaSHV gene sequence was performed using PCR**.**

TARGET GENE	PRIMER	LENGTH
***bla*SHV**	**Forward-5; -TTATCTCCCTGTTAGCCACC-3'** **Reverse- 5' - GATTTGCTGATTTCGCTCGG-3'**	**795 [16]**

Table 1: Primers used for *bla*-SHV gene

***bla*-TEM gene**

TARGET GENE	PRIMER	LENGTH
***bla*TEM**	**Forward-5' -ATGAGTATTCAACATTTCCGTG-3'** **Reverse-5' -TTACCAATGCTTAATCAGTGAG-3'**	**861 [16]**

Table 2: Primers used for *bla*-TEM gene

Polymerase Chain Reaction (PCR)

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water.

The cyclic conditions for blaTEM gene, initial denaturation at 95 ℃ for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

Step	**Program** **blaTEM** **Time**	**Temperature**	**Cycles**
Initial denaturation	15 min	95 ºC	
Denaturation	30 s	94 ºC	
Annealing	1 min30 s	59 ºC	30
Extension	1 min 30 s	72º C	
Final extension	10 min	72º C	

Table No. 3 : The PCR cycling conditions to amplify blaTEMgene fragments.

Step	Program blaSHV Time	Temperature	Cycles
Initial denaturation	15 min	95 ºC	
Denaturation	30 s	94 ºC	
Annealing	1min 30 s	52 ºC	30
Extension	1 min 30 s	72º C	
Final extension	1 min 30 s	72º C	

Table No. 4 : The PCR cycling conditions to amplify blaSHVgene fragments

For acquired blaSHV genes, the initial denaturation was at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 52 °C for 1 min 30 s and 72 °C for 1 min 30 s, followed by extension of 72 °C for 1 min 30 s.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

- The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments.
- The resulting PCR product was subjected to 1 % agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA).
- A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample.

STATISTIC ANALYSIS

Data along with statistic was recorded by the Microsoft Excel. The values were represented in Numbers percentage and bar diagram..

RESULTS

Throughout the learning period (August 2022 to April 2024), A total 836 urine samples were collected and processed. Out of the 900 isolates there were 366 (40.6%) which showed the isolates of *E. coli*. In which 82 (22.5%) were phenotypically identified as ESBL producers and 284 (77.6%) were Non-ESBL (Fig. 1).

Fig 4: Distribution of E.coli Isolates among ESBL / Non-ESBL

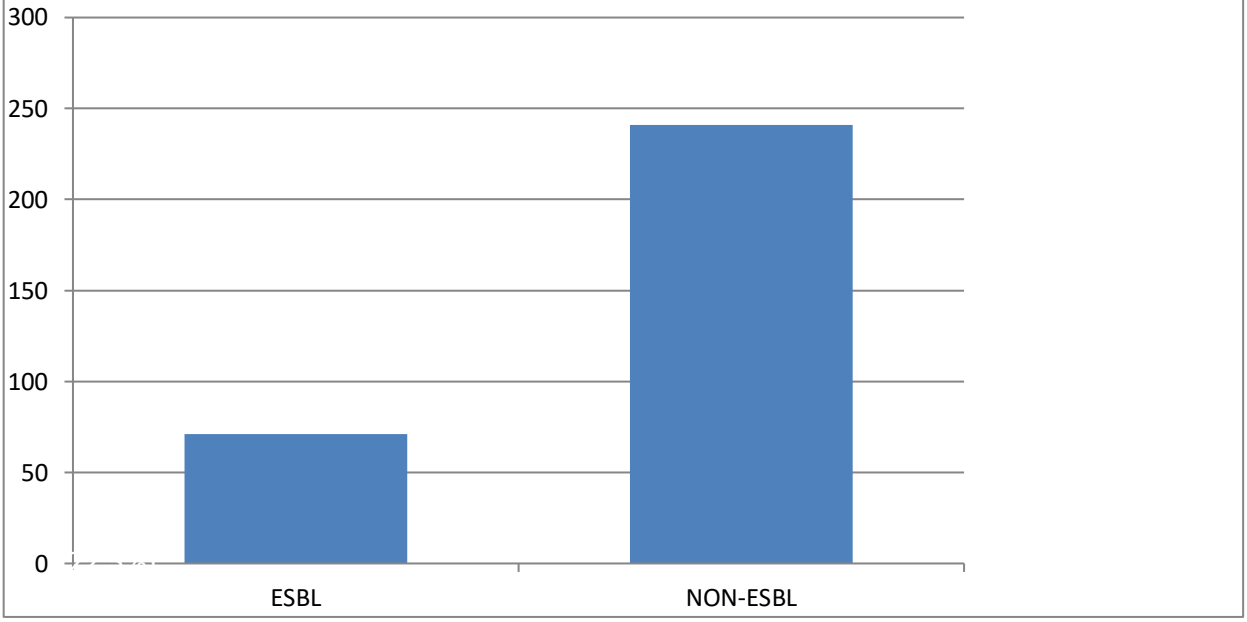


Table 5 shows that Out of 366 patients who were included in this study 110 (30%) were Male & 256 (69.9%) were Female patients.

Table 5: Distribution of Patients according to Gender (Male/ Female)

Gender	No. of Isolates	Percentage (%)
Male	110	30%
Female	256	69.9%
Total	366	100

Table 2 shows that the age group of 21–30 years old accounts for the greatest number of instances (24.1%), while the age group of patients over 80 years old accounts for the fewest (0.9%).

Table 6: Distribution of Patients according to Age group

Age Group	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	Total
No. of Isolates	37	40	88	54	50	56	23	14	3	366
Percentage	10.2%	10.7%	24.1%	15.1%	13.5%	15.3%	6.3%	3.9%	0.9%	100%

Table 6 shows that the overall susceptibility of ESBL isolates to various antibiotics was as Follows: Ampicillin(17.20%), Ampicillin/Sulbactam (28.5%), Gentamycin (65.7%), Cefoxitin (51.0%), Amikacin (80%), Ciprofloxacin (48%), Meropenem (97.2%), Ceftazidime(0%), Ceftazidime/ clavunalate(100%), Piperacillin/tazobactam (85.7%), Ceftriaxone(0%), Nitrofurantoin(100%), Tigecycillin(97.2%) and fosfomycin(97.2%).

Table no. 7 Antimicrobial Sensitivity & Resistivity of ESBL producing E. coli

Antibiotics	**Sensitivity**	**Resistivity**
Ceftazidime/clavunalte	100%	0%
Nitrofurantoin	100%	0%
Fosfomycin	97.20%	2.80%
Meropenem	97.20%	2.80%
Tigecycillin	97.20%	2.80%
Piperacillin/Tazobactam	85.70%	14.30%
Amikacin	80%	20%
Gentamycin	65.70%	34.30%
Cefoxitin	51%	49%
Ciprofloxacin	48%	52%

Ampicillin/Sulbactum	28.50%	71.50%
Ampicillin/Sulbactum	17.20%	82.80%
Ceftazime	0%	100%
Ceftriaxone	0%	100%

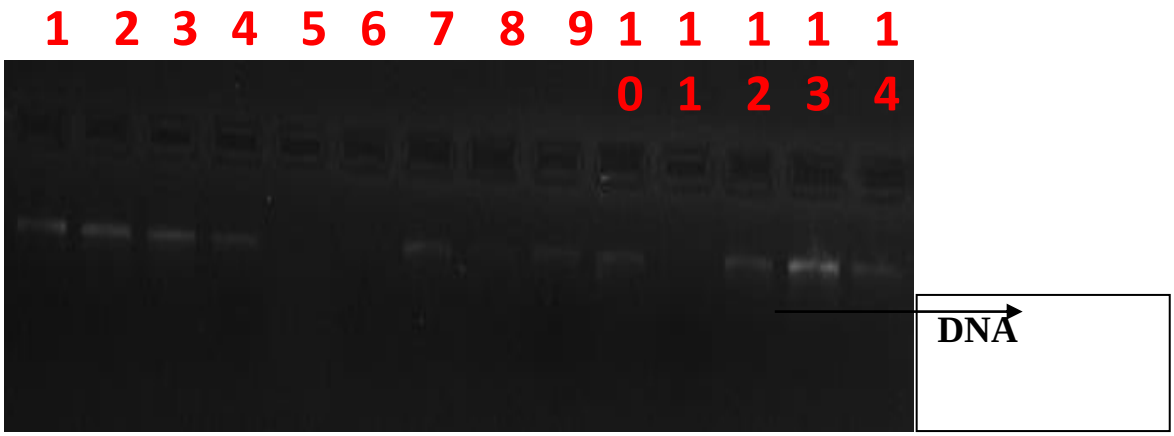
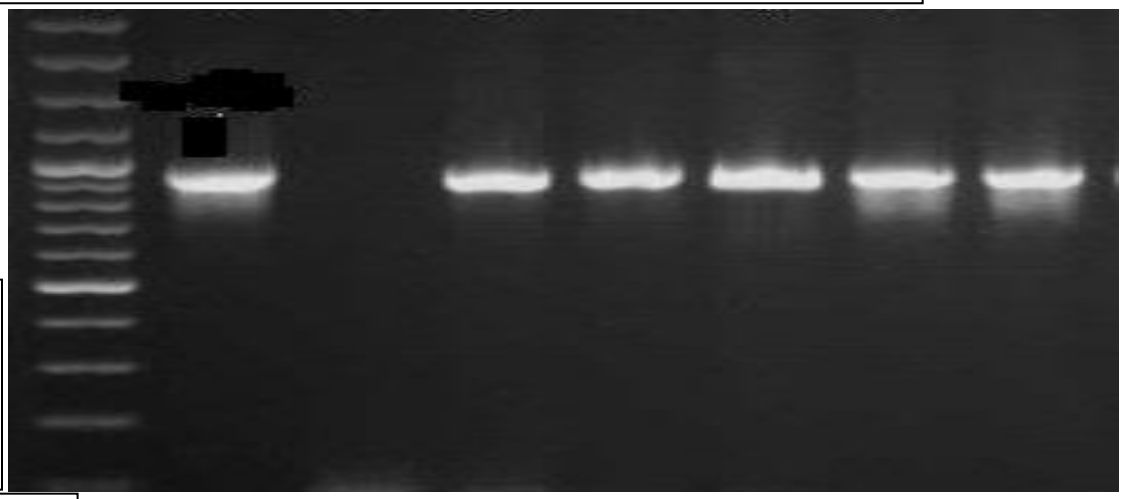


Fig No. 5: The DNA extraction for *bla*-TEM gene

L; Ladder; L1,L2,L3,L4,L5,L6,L7



DNA Ladder

861 BP

Fig No. 6 : The *bla*TEM 861 bp

L corresponds to the DNA Ladder; L1 corresponds to the positive Control; L2 Corresponds to the Negative

Control to *bla*TEM gene; L3-L7are the sample positive for*bla*TEM gene

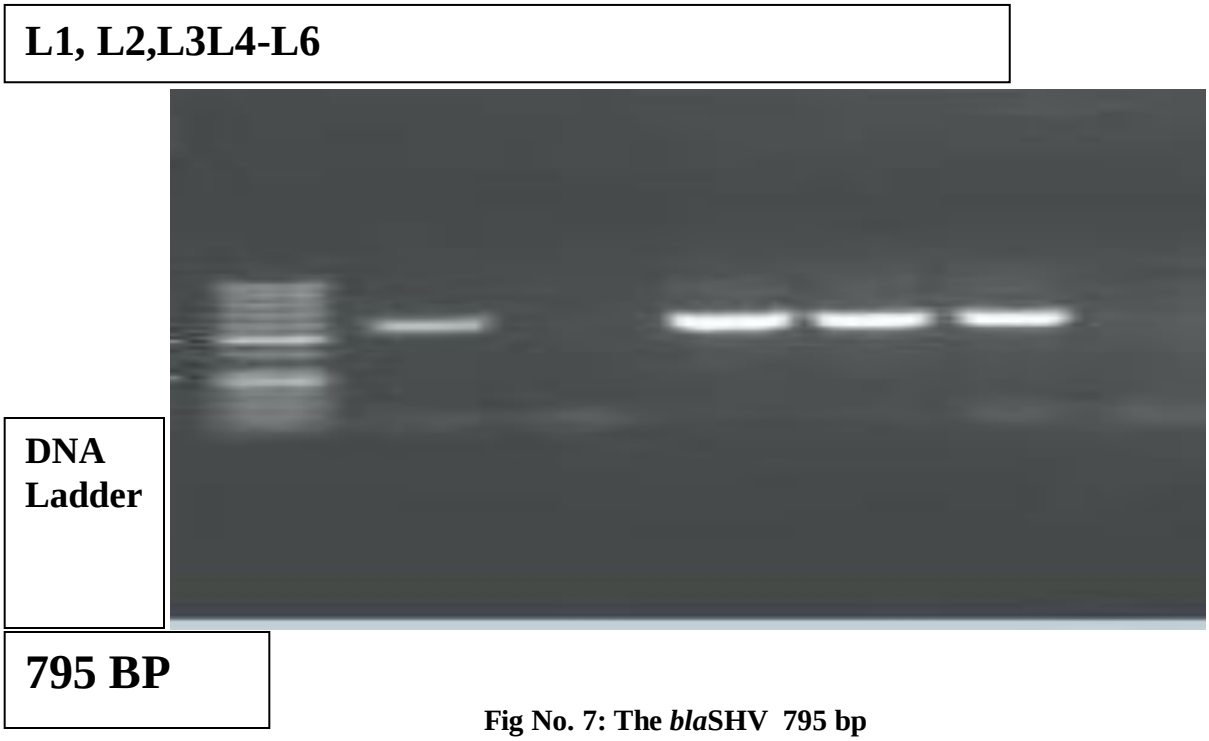


Fig No. 7: The *bla*SHV 795 bp

L1 corresponds to the DNA Ladder; L2 corresponds to the positive Control; L3 Corresponds to the Negative Control to *bla*SHV gene; L4-L6are the sample positive for*bla*SHV gene

Table No. 8: Distribution of different genes in ESBL-producing *E.coli* isolates

Gene	No. of Gene Detected	Percentage
blaTEM	40	48.7%
blaSHV	11	13.4%

In the current study out of the total 82 isolates there were 40 (48.7%) observed positive for blaTEM gene and 11 (13.4%) observed positive for blaSHV gene.

DISCUSSION

Over the past 20 years, gram-negative bacteria that produce ESBL in particular, *E. coli*—have emerged as significant

global pathogens in both community-acquired and hospital-acquired illnesses. It is advised to use β-lactam medications, such as carbapenems and long spectrum cephalosporins, to treat enterobacterial infections There were initially only a few bacterial species, but they are now rapidly expanding, and the maturity of conflict to extended-spectrum cephalosporins in Gram-negative bacteria has been a major cause for concern [17,18].
Characteristic of antimicrobial resistance are the differences between regions, hospitals and even departments. *E. coli* strains are susceptible to commonly used antimicrobial agents in treatment of UTIs. However, antibiotic resistance of uropathogenic *E. coli* in UTIs is increasing worldwide [18].

In the present study the detection of the spread of ceftazidime antibiotic resistance in isolated *E. coli* from urine culture were observed. The population of *E. coli* that was identified showed a significant resistance to commonly used antibiotics. In the present study's findings, disc diffusion tests revealed that 22.5% of *E. coli* samples produced ESBL.Various other studies from India have reported high ESBL production ranging from 41% to 63.5% [17-19]. In Tehran 60%, In Pakistan 41%, In Iran 44.4%. [20-21]. There was a study performed by the other research investigator where among *E. coli* bacteria of antibiotic susceptibility pattern of ESBL Isolates revealed poor susceptibility to Ampicillin (9.2%), cefoxitin (34.4%), Ampicillin/Sulbactam (35.5%), while susceptibility to Nitrofurantoin (77.6%), Amikacin (73.3%), Meropenem (72.1%) [17, 18]. Similarly, In this study ESBL producing *E. coli* shows 100% sensitivity in Ceftazidime/ Clavunalate, Nitrofurantoin followed by Fosfomycin, Meropenem & Amikacin. [17,22-24]
This study shows that out of 366 patients 82 (22.5%) ESBL 284 (77.5%) & Non-ESBL. And this study also observed that maximum number of patients belong to age group of 21–30 years old 88 (24.1%) followed by 31-40 years. It was also observed that Female were more affected 256 (69.9%) than Male 110 (30%) . This study was parallel to the study performed by the other research investigator where UTIs caused by ESBL-producing *E. coli* were overall far more common among females [25,26].

In the present study it was observed that out of the total 82 isolates there were 40 (48.7%) observed positive for blaTEM gene and 11 (13.4%) observed positive for blaSHV gene. This study was in accordance to the study performed by the other research investigator M.C. El bouamri et al., where the ESBL production patterns observed included single production of CTX-M (70%), SHV (12%) but in constrast with TEM (0%) [27]. There was another study which was in support to the current study where the $bla_{\text{CTX-M}}$ (77.4%; n = 377), bla_{TEM} (54.4%; n = 265) and *aac(6')-Ib-cr* (52%; n = 253) genes and a low proportion of bla_{SHV} and *qnr* genes were observed [28].
Antimicrobial resistance and the growth of multidrug-resistant *E. coli* strains pose a danger to the efficient management of UTIs, resulting in higher rates of morbidity, longer hospital stays, higher treatment costs, and death from disease.
Enterobacterales producing ESBL persist as most recurrent cause of expanded-spectrum cephalosporin resistance in these kind of infections. E. coli that producing ESBL primarily causes UTIs, although more serious infections can also happen. More research examining the risk factors, diagnostic importance, and possible treatments for acquired in the community illnesses triggered by these microbes is required because there are relatively only a few alternatives for treating these infections [28]. However, these patients usually delay receiving the proper therapy, which could have negative clinical results. The fact is that we have a good arsenal at our disposal to treat these infections, despite recent disagreements over if a carbapenem antibiotic has to be employed for treating severe infections caused by ESBLs or while certain β-lactamases/ β-lactamases inhibitor combinations remain suitable. It continues to remain crucial for us to keep a close eye on ESBLs in patients isolates as well as in monitoring because they have become widespread in healthcare isolates of Enterobacterales [29].

Antibiotic resistance is one of the most serious global health problems and threatens the effective treatment of bacterial

infections. Of greatest concern are infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC).

[30,31]. Therefore, early identification of this organism's antimicrobial resistance traits in a given area will aid in the prompt adaptation of measures that will lessen the possibility of antimicrobial agent misuse and stop the establishment and subsequent spread of such MDR isolates [29]. These findings should alert underdeveloped nations and Indian medical authorities to the grave implications of rising antibiotic resistance. As a result, it's critical to step up efforts to keep an eye on and stop the spread of antibiotic resistant bacteria in hospitals and the community.

Conclusion

The management of ESBL infections is significantly hampered by high antibiotic drug resistance, which forces physicians to use carbapenems and colistin more frequently. TEM, CTX-M-1, CTX-M-15, and SHV-type ESBL are quite prevalent, and lesser varieties such as OXA-1, VEB, and PER-2 type β-lactamase are emerging. The spread of ESBL justifies the need for more thorough monitoring in order to implement suitable control measures. The use of infection control bundles and checklists in hospitals is critically needed. To help choose the best antibiotics for empirical therapies, local antibiograms are crucial.

Declarations:

Conflicts of interest: There is not any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

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