TO STUDY THE MOLECULAR PROFILING AND ITS ASSOCIATION TO EXTENDED SPECTRUM B-LACTAMASE (ESBL) PRODUCING *E.COLI* ISOLATES WITH SPECIAL REFERENCE TO *blaTEM , bla*CTX AND *blaSHV* GENE FROM PATIENTS OF URINARY TRACT.

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KEYWORDS
ESBL, UTI, MDR, β-Lactams , CLSI,DNA, PCR, blaCTX, blaTEM, blaSHV

ABSTRACT

Introduction: Longer hospital stays, higher treatment costs, and fewer treatment options—particularly wide-spectrum antibiotics—are often linked to the rise of extended-spectrum β-lactamase (ESBL). Among the most commonly given antibiotics in human medicine are β-lactams. However, resistance to these drugs has increased dramatically due to their widespread and mostly improper use, especially as a result of the development of extended-spectrum β-lactamase (ESBL).

Aim and Objective: To study the molecular characterization of extended spectrum β-lactamase (ESBL) producing *E.coli* isolates with special reference to *blaTEM*, bla*CTX* 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, November 2023 to November 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*, *blaCTX* and *blaSHV* was detected using the PCR.

Results: In the present study out of the 1012 isolates there were 366 (35.5

which showed the growth of *E. coli*. In which 82 (22.4%) were phenotypica identified as ESBL producers and 284 (77.6%) were Non-ESBL. Out of 366 isolates, 110 (30%) were Males and 256 (69.9%) were Females patie The overall susceptibility of ESBL isolates to various antibiotics was Ampicillin (17.20%), Ampicillin/Sulbactam (28.5%), Gentamycin (65.7 Cefoxitin (51.0%), Amikacin (80%), Ciprofloxacin (48%), Meropen (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 total 82 isolates there were 42 (51.2%) observed positive for blaTEM gene, (29.2%) observed positive for blaCTX and 13 (15.8%) observed positive blaSHV gene.

Conclusion: There are now fewer treatment choices and increased medical costs as a result of the substantial expansion of E. coli that produces ESBL. Due to the significant growth of E. coli that produces ESBL, there are now fewer options for treatment and higher medical expenses. To assist clinical care management, efficient infection control, and appropriate antibiotic treatment, trends for regional epidemiological data on antimicrobial resistance need to be updated.

INTRODUCTION

Antibiotics are the first drugs of choice to treat infectious diseases. A rise in infectious diseases, increasing rate of drug resistance, and indiscriminate use of antibiotics are the reasons behind the high usage of antibiotics in developing countries [1,2]. Antimicrobial resistance (AMR) has a negative impact on achieving Sustainable Development Goals (SDG), food safety, and food security. In the antimicrobial resistance (AMR) era, the evolving resistance caused by extended-spectrum β-lactamases (ESBLs) led to higher morbidity, prolonged hospital stays, and expensive treatment options [2].

The rise of antimicrobial resistance, particularly from extended-spectrum β-lactamase producing *Enterobacteriaceae* (ESBL-E), poses a significant global health challenge as it frequently causes the failure of empirical antibiotic therapy, leading to morbidity and mortality [3].

ESBLs are Gram-negative bacteria of the *Enterobacteriaceae* family that carry ESBL genes in their plasmids or chromosomes, produce β-lactam hydrolyzing enzymes, and are rightly considered to be among the most challenging pathogens by the World Health Organization (WHO) [4]. ESBL-producing *Enterobacteriaceae* (ESBL-E) confer resistance to penicillin—in addition to aztreonam and first-, second-, and third-generation cephalosporins—but are unable to hydrolyze cephamycin or carbapenems [5,6]. Carbapenem has been the drug of first choice for treating ESBL-E-induced infection for a long time . This is changing, though, due to many factors including the recent emergence of carbapenemase-producing bacteria. Thus, there is an urgent need to develop alternative approaches.

The ESBL-encoding genes are highly diverse in nature and can be classified into many families with unique characteristics such as *bla*TEM, *bla*SHV, and *bla*CTX-M. TEM 1, the first plasmid and transposon-mediated β-lactamase, was isolated from the blood culture of a named Temoniera in Greece in the early 1960s [7] . It has spread worldwide and is now found in many species of the family *Enterobacteriaceae*, *P. aeruginosa*, *Hemophilus influenzae*, and *Neisseria gonorrhoeae* . The SHV-1 type is common in *Klebsiella* spp. and *E. coli* . CTX-M-type ESBL are predominant in *E. coli*, *K. pneumoniae*, *S. enterica* serovar *Typhimurium*, and *Shigella* spp. The plasmid-mediated OXA and AmpC-type ESBL were discovered in *P. aeruginosa* and *K. pneumoniae* isolates, respectively [8].

ESBLs make it difficult to treat infections in acute critical care settings and are most commonly seen in Enterobacteriaceae in India. In India as well as other countries, CTX-M, TEM, and SHV-type ESBL are now common, and hospital-associated pathogenic bacterial strains are expressing more of these enzymes, which could spread widely. ESBLs from a number of nations have also been described, including OXA-1, PER-type, GES-type, and VEB-type. Using various techniques to identify ESBL resistance, many researchers in India have reported prevalences ranging from 7.0 to 91% [9] .

It is important to note that some β-lactamases may not be inactivated by some classical inhibitors such as clavulanate acid, sulbactam, and tazobactam [10]. Mechanisms of resistance in Gram-negative bacteria may also involve reduced membrane permeability through genomic mutations, decreased amounts of β-lactam antibiotics that can enter the cell, and a marked increase in antibiotic reflux from the periplasm to the exterior of the cell [11]. 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 [12].

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 [13].

Compared to traditional phenotypic methods, polymerase chain reaction (PCR)-based molecular techniques are quick, precise, and offer higher sensitivities for identifying ESBL-resistant genes. In addition to providing practitioners with a focused treatment strategy, they also aid in epidemic containment and infection control policy implementation.

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 [14].

Moreover, there is an urgent need to develop precise diagnostic tools, new drugs, and novel strategies against difficult-to-treat antibiotic-resistant pathogens, including the use of antibiotics in combination or with adjuvants, bacteriophages, antimicrobial peptides, nanoparticles, antibacterial antibodies, and photodynamic light therapy

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* , *bla CTX* 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, November 2023 to November 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 [15].

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, *bla*CTX 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, blaCTX 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, blaCTX 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

***bla*-CTX gene**

TARGET GENE	PRIMER	LENGTH
***bla*CTX**	**Forward- 5' -SCSATGTGCAGYACCAGTAA-3'** **Reverse-5' -CCGCRATATGRTTGGTGGTG-3'**	**544 [16]**

Table 3: Primers used for *bla*-CTX 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 °C 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. 4 : The PCR cycling conditions to amplify blaTEM gene 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. 5 : The PCR cycling conditions to amplify blaSHV gene fragments

Step	Program blaCTX 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. 6 : The PCR cycling conditions to amplify blaCTX gene 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 cyclic conditions for blaCTX gene, initial denaturation at 95 °C 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 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

In the present study out of the 1030 isolates there were 366 (35.5%) which showed the growth of *E. coli*. In which 82 (22.5%) were phenotypically identified as ESBL producers and 284 (77.6%) were Non-ESBL. Out of the 366 isolates, 110 (30%) were Males and 256 (69.9%) were Females patients.

Table 7 shows that Out of 366 patients who were included in this study 110 (30%) were Male & 256 (69.9%) were Female patients.

Table 7: Distribution of Patients according to Gender (Male/ Female)

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

Table 8 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 8: 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 9 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 9: 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%

Figure No 4.: The DNA Extraction of the test isolates

L1, L2,L3L4-L6

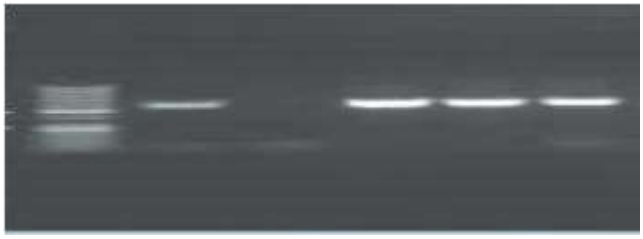

Figure No. 5: The *bla*SHV 795 bp

L1 corresponds to the DNA Ladder; L2 corresponds to the positive Control; L3 Corresponds to the Negative Control to *bla*SHV gene; L4-L6 are the sample positive for *bla*SHV gene

***bla*CTX**
DNA Ladder
L1-L9,L10,L11-16,L19,20

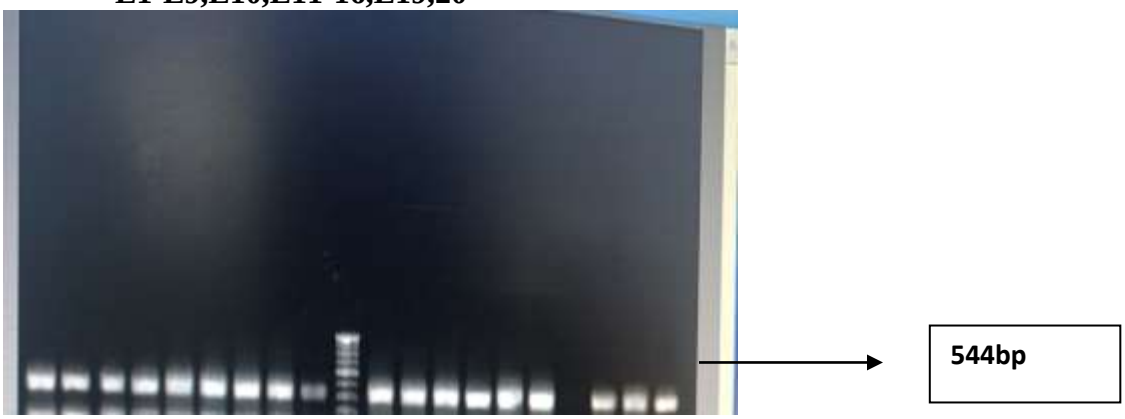

Figure No. 6 : The Amplified DNA with PCR for blaCTX gene of *E.coli* . Lane -1-9 are positive for CTX gene; Lane 10 is the DNA Ladder; Lane 11-16 and 19, 20 are CTX gene positive; Lane 17 is the Negative control for CTX gene; Lane 18 is the positive control for CTX gene

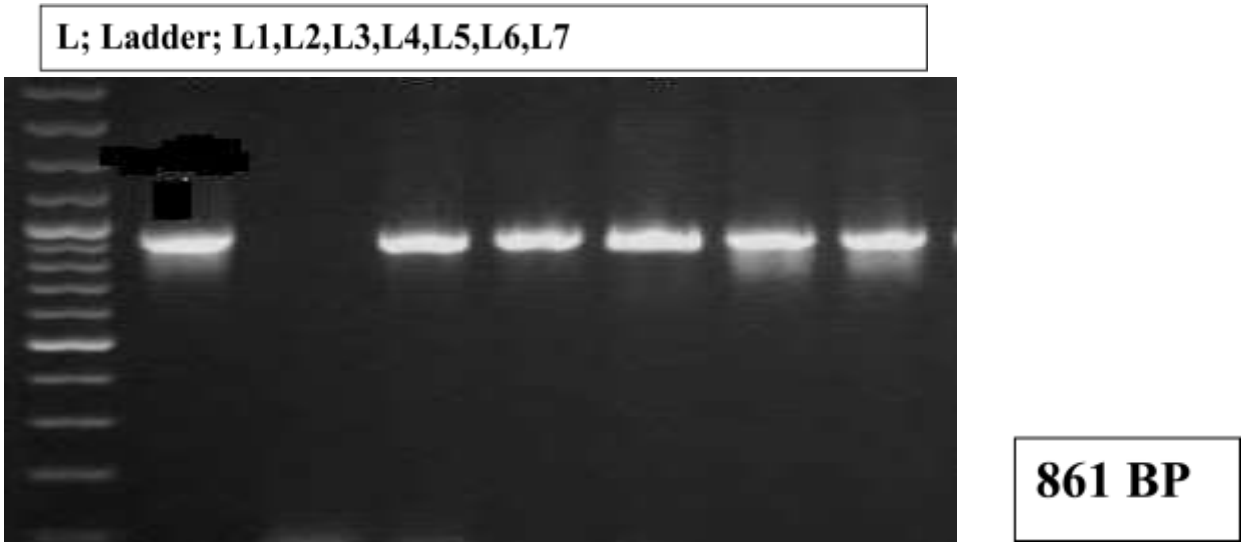

Figure No. 7 : The *bla*TEM 861 bp
L corresponds to the DNA Ladder; L1 corresponds to the positive Control; L2 Corresponds to the Negative Control to *bla*TEM gene; L3-L7 are the sample positive for *bla*TEM gene

Table 10: Distribution of different genes in ESBL-producing *E.coli* isolates

Gene	No. of Gene Detected	Percentage
blaCTX	**24**	**29.2%**
blaSHV	**13**	**15.8%**
blaTEM	**42**	**51.2%**

In the current study out of the total 82 isolates there were 42 (51.2%) observed positive for blaTEM gene, 24 (29.2%) observed positive for blaCTX and 13 (15.8%) observed positive for blaSHV gene 13`(15.8%) .

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].

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].

This study shows that out of 366 patients 82 (22.4%) 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 [19,20].

In the present study it was observed that out of the total 82 isolates there were 42 (51.2%) observed positive for blaTEM gene, 24 (29.2%) observed positive for blaCTX and 13 (15.8%) 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%) [21]. There was another study which was in support to the current study where the bla CTX-M (77.4%; n = 377), bla TEM (54.4%; n = 265) and Ib-cr (52%; n = 253) genes and a low proportion of bla SHV and qnr genes were observed [22].

There was another study by Sheetal verma et al., in 2022 where phenotypically positive ESBL isolates, blaTEM (49.4%) was the most common genotype followed by blaCTX- M1 (31.97%), blaOXA-1 (30.1%), and blaSHV(11.9%) either alone or in combination. This study was in support to the present study [23]. In a study from Assam, CTX-M, TEM, and SHV were detected in 54.4, 33.9, and 15.4% isolates, respectively [24]. There was another study was in alignment to the current study where the blaCTX−M−1 (60.7%) was the most common among [25]. There was another study which was in accordance to the current study where out of the collected strains of ESBL-producing E. coli, had 81% blaTEM, 16.2% blaSHV, and 32.4% blaCTX-M genes. Similarly, 64.7% blaTEM, 35.2% blaSHV, and 41.1% blaCTX-M genes existed in the isolates of *K. pneumoniae* [26].

There were few studies from Central India which were also in accordance to the current study and reported blaTEM gene most predominant followed by blaCTX-M and blaSHV [27].

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 . 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.

Common ESBL genes coding for isolates of K. pneumoniae and E. coli were determined as CTX-M (cefotaximase that preferentially hydrolyzes cefotaxime), TEM (found and isolated in the early 80s from Teminora who was a Greek patient), and SHV (for variable of sulphydryl which was first observed in a single Klebsiella ozaenae strain retrieved in Germany) [28-30] . These genes which are mediated by transposons, plasmids, or chromosomes are all sporadically described all over the world.

CONCLUSION

High antibiotic drug resistance makes it difficult to treat ESBL infections and pushes doctors to use colistin and carbapenems more often. OXA-1, VEB, and PER-2 type β-lactamase are emerging, while TEM, CTX-M-1, CTX-M-15, and SHV-type ESBL are rather common. The proliferation of ESBL makes more careful monitoring necessary to put appropriate control mechanisms in place.
Hospitals urgently need to adopt infection control bundles and checklists. Local antibiograms are essential for selecting the most effective antibiotics for empirical treatments.

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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