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To Study The Molecular Characterization Of Extended Spectrum B-Lactamase (ESBL) Producing E.COLI Isolates With Its Antibiotic Resistance Profile to Blactx and Blashv Gene From Patients of Urinary Tract Infections

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ABSTRACT

Introduction: Health care-associated urinary tract infections (HAUTIs) are common and contribute to severe morbidity and high fatality rate in hospitalized patients. About 80% of HAUTIs occur in patients who undergo instrumentation of the urinary tract including catheter-associated urinary tract infections. The emergence of extended-spectrum β-lactamase (ESBL) is frequently associated with prolonged hospital stay, increased treatment cost, and limited treatment options especially wide-spectrum antibiotics. However, due to their widespread and mostly inappropriate usage, resistance to these medications has significantly grown, particularly as a result of the creation 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 associated gene to *blaCTX* and *blaSHV* gene from patients of urinary tract infections.

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

Results: In the present study out of the 1012 isolates there were 366 (36.1%) 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. 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 there were 25 (30.4%) were observed positive for blaCTX gene and 11 (13.4%) observed positive for 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. Trends for regional epidemiological data on antimicrobial resistance must be updated to support appropriate antibiotic administration, effective infection control, and clinical care management.

Keywords: *ESBL, Urinary Tract Infection, MDR, β-Lactams, CLSI*

1. INTRODUCTION

Urinary tract infections (UTIs) are a common source of illnesses linked to healthcare and the general public, particularly in India [1, 2]. Thirty to fifty percent of adults have urinary tract infections, which can lead to illnesses. Numerous risk factors, including prior hospitalisation, immunosuppressive medication usage, diabetes, urinary tract obstructions, surgical manipulation, catheterisation, and other co-morbidities, are associated with this high incidence [3].

Seventy to eighty percent of UTIs are caused by *Escherichia coli* [4]. Treating UTI patients has become increasingly challenging due to the rapid rise in antibiotic resistance [5]. The development of the extended-spectrum β-lactamase (ESBL) enzyme by *E. coli* is a significant problem, but the rapid increase in fluoroquinolone and aminoglycoside resistance has also had a significant impact on the limited and challenging treatment options accessible to infected individuals [6,7]. Numerous studies have demonstrated that *E. coli*, which produces ESBLs, is responsible for a sizable percentage of UTIs in hospitals and communities [8].

The emergence of extended-spectrum β-lactamase (ESBL) is frequently associated with prolonged hospital stay, increased treatment cost, and limited treatment options especially wide-spectrum antibiotics [9,10].

Resistance to third-generation cephalosporins (e.g., cefotaxime and ceftazidime) by the production of extendedspectrum β-lactamase (ESBL) enzymes (mainly blaCTX-M, blaTEM, and blaSHV genes) among Enterobacteriaceae, including *E. coli* has been identified as a critical emerging issue of public health concern by the World Health Organization (World Health Organisation, 2017) [11].

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-ceplalosporins ceftazidine and cefotaxim are frequently utilised. These more recent medications were hydrolysed as a result of the creation of novel β-lactames [13].

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% [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].

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. Thus, the current investigation was conducted to examine the molecular characterisation of E. Coli isolates that produce extended spectrum β-lactamase (ESBL), with particular attention to the blaCTX and blaSHV genes from urinary tract patients.

2. MATERIAL AND METHODS

This Cross sectional study was carried out in the Department of Microbiology for a period of 12 months i.e, August 2023 to August 2024 at a tertiary care centre. A total of 366 *E. coli* isolates of all the Uropathogenic *E. coli* strain which were isolated from urine samples were collected from hospitalized and consultation patients in the study. The Antimicrobial susceptibility testing was performed according to the CLSI guidelines 2024 and antimicrobial susceptibility of novel beta-lactum / beta-lactamases inhibitor combination drugs (ceftazidime-avibactam) was performed [15]. To detect Extended spectrum beta lactamases in *E. coli* isolates from urinary tract infection by phenotypic method combination dics diffusion test.

Inclusion Criteria:

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

Exclusion Criteria:

- Patient who refused to give consent were excluded.

3. 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 2024 criteria.

4. 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).

5. 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.

6. GENOTYPIC METHOD

MOLECULAR METHODS: For the detection of the gene 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 blaSHV gene

Fig No.3: Primers of blaCTX gene

Polymerase Chain Reaction (PCR)

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

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

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

***bla*-CTX gene**

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

Table 2: 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 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 PCR cycling conditions

Step	Program blaCTX 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 3 : The PCR cycling conditions to amplify blaCTX 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 4 : The PCR cycling conditions to amplify blaSHV 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 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.

7. STATASTICAL ANALYSIS

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

8. RESULTS

In the present study out of the 1012 isolates there were 366 (36.1%) 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 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%

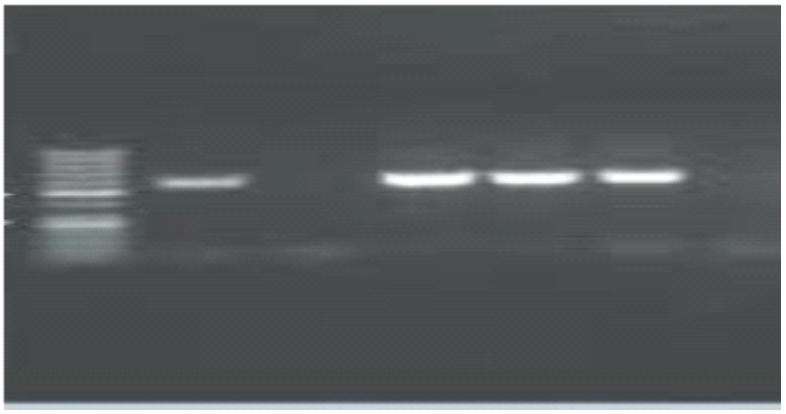
Table 6 shows that the overall susceptibility of ESBL isolates to various antibiotics was as Follows: Ampicillin(17.20%), Ampicillin/Sulbactam (28.5%), Gentamycin (65.7%), Cefoxitin (51.0%), Amikacin (80%), Ciprofloxacin (48%), Meropenem (97.2%), Ceftazidime(0%), Ceftazidime/ clavunalate(100%), Piperacillin/tazobactam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

L1, L2,L3L4-L6



795 BP

Fig No. 4: 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 DNA

Figure No 5.: The DNA Extraction of the CTX gene

***bla*CTX**

544 BP

DNA Ladder

L1-L9,L10,L11-16,L19,20

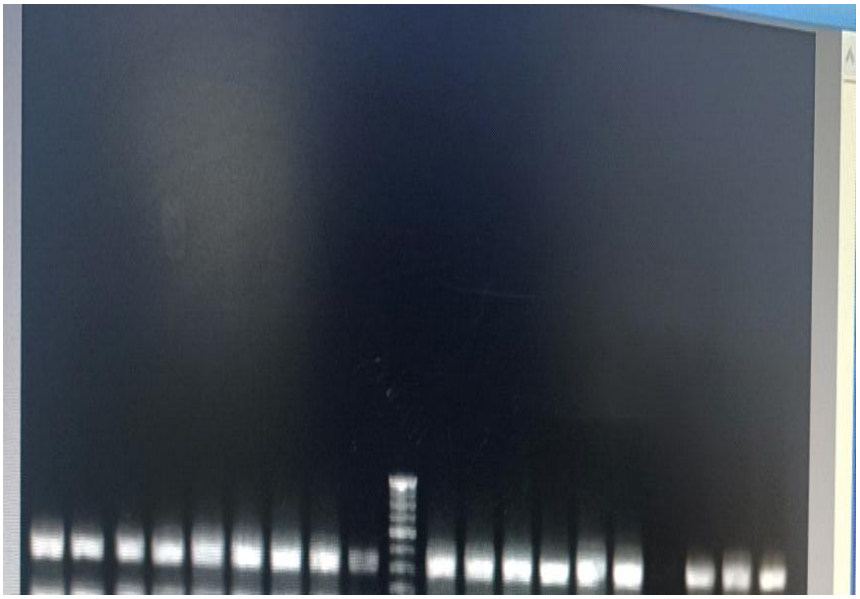


Figure No. 6 : The Amplified DNA with PCR for blaCTX gene of *E.coli* . Lane -1-9 are positive for CTX gene; Lane 10 is the DNA Ladder; Lane 11-16 and 19, 20 are CTX gene positive; Lane 17 is the Negative control for CTX gene; Lane 18 is the positive control for CTX gene

Table 8: Distribution of different genes in ESBL-producing *E.coli* isolates

Gene	No. of Gene Detected	Percentage
blaCTX	**25**	**30.4%**
blaSHV	**11**	**13.4%**

In the current study out of the total 82 isolates there were 25 (30.4%) were observed positive for blaCTX gene and 11 (13.4%) observed positive for blaSHV gene.

9. DISCUSSION

Over the last four decades, extended-spectrum β-lactamases (ESBLs) have presented a major challenge to treating infections caused by Enterobacterales in health care and community settings. Resistance to third- and fourth-generation cephalosporins mediated by ESBLs and the often associated coresistance to other antibiotic classes, such as fluoroquinolones, aminoglycosides, and sulfonamides, have led to the increased use of carbapenems worldwide, which in turn is linked to increasing resistance to these agents [17,18]. Extended Spectrum Beta-Lactamases (ESBLs) encoding genes (TEM, SHV and OXA) were amplified from multidrug resistance *E. coli*. The multidrug resistance *E. coli* isolates from different clinical sources were documented to be plasmid encoded and resistance against *β*-lactam and cephalosporin.

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].

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 [29]. In a study from Assam, CTX-M, TEM, and SHV were detected in 54.4, 33.9, and 15.4% isolates, respectively [30]. There was another study was in alignment to the current study where the blaCTX−M−1 (60.7%) was the most common among [31]. 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* [32].

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 [33].

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 . 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 .

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).

[34]. 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 [35,36,37]. 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.

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) [38-40] . These genes which are mediated by transposons, plasmids, or chromosomes are all sporadically described all over the world.

Because there is an increase in the rates of bacterial resistance every year, leading to rising global concern, it is highly significant to understand susceptibility patterns as hospital stays may prolong and mortality rates increase due to inappropriate empirical antimicrobial therapy, which can be controlled given appropriate therapy.

10. CONCLUSION

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). 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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