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Antimicrobial Gene Profile of Extended Spectrum Beta Lactamases *Escherichia coli* from Human Clinical Isolates in North India

Hamid Ahmad Shah[1*], Dalip K. Kakru[1] and Mohd Altaf Bhat[2]

[1]*Department of Microbiology, SMSR, Sharda University, Greater Noida, U.P., India*
[2]*Department of Veterinary Microbiology, FVSc & AH, SKUAST-K, Kashmir, India*

**Corresponding author*

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A B S T R A C T

Antimicrobial resistance (AMR) is an extensive public health problem due to the rapid increase in the population of multidrug-resistant bacteria. *Escherichia coli,* a common bacterial pathogen, has demonstrated resistance to beta-lactam antibiotics through the formation of beta-lactamase enzymes. Understanding the genetic diversity and co-carriages of β-lactamase genes in *E. coli* is essential for developing effective strategies to combat AMR. In this study, we conducted a comprehensive molecular characterization of beta-lactamase *E. coli* isolates collected from clinical in the northern Indian region. To find and categorize beta-lactamase genes, 210 *E. coli* isolates were submitted for phenotypic and genotypic investigation. Different beta-lactamase gene variations were detected using DNA sequencing and polymerase chain reaction (PCR). They were further characterized on the basis of antimicrobial gene profile. The present study findings revealed a diverse range of beta-lactamase gene variants in the *E. coli* isolates, including SHV, TEM, and CTX-M types. Importantly, we seen a greatexistence of extended-spectrum beta- lactamase (ESBL)-making the strains of *E. coli,* indicating the growing concern of ESBL-mediated resistance in the region.Antimicrobial gene profile revealed *tetA* present in 53.22% of isolates whereas t*etB*is present in 32.24% isolates Similarly *Sul1* and *Sul2* is present in 8.1% and 16.52% respectively.None of the isolates showed the presence of *Dfrla*and *AadA* genes. This research provides valuable awareness of the molecular epidemiology of beta-lactamase gene variants in *E. coli* in the northern Indian part, highlighting the urgent need for targeted interventions to curb the spread of antimicrobial resistance. Understanding the genetic diversity and mechanisms of resistance is crucial for the development of effective antibiotic stewardship programs and the design of novel therapeutic approaches to combat AMR in this region.

Introduction

Antibiotic resistance is a pathogenic bacterial trait that permits bacteria to survive and proliferate in the presence of therapeutic levels of antibiotics that inhibit or kill them. Antibiotic-resistant bacteria can therefore evade the effects of medication and proliferate, posing a major risk to public health. Drug resistance is spreading at an alarming rate, providing severe challenges in the treatment of infectious diseases caused by multidrug-

resistant bacteria (MDR bacteria). Multidrug-resistant bacterial infections are associated with a high rate of morbidity, death, high healthcare costs, and restricted therapy alternatives. As a result, detecting MDR bacteria is critical for determining therapeutics and patient isolation, both of which are required to avoid the transmission of these pathogens as well as community-acquired (nosocomial) infections and epidemics (Sousa *et al.,* 2004; Paterson and Bonomo, 2005). Major public health concern globally nowadays is antibiotic resistance caused by enteric bacteria (WHO, 2020).

The usage of beta-lactam antibiotics has increased which has resulted in an increase in Enterobacteriaceae resistance because of the formation of the β-lactamases which act on the beta-lactam ring of antibiotics. ESBL are a subset of β-lactamases generated by gram-negative bacteria, primarily *Enterobacteriaceae* and *Pseudomonas aueruginosa* (Chaudhary and Aggarwal, 2004). The most common strains that produce ESBLs. are *Klebsiella* & *E. coli* (Jacoby and Medeiros, 1991; Tzelepi *et al.,* 2000). In hospital settings, the rise of *E. coli* beta-lactamases is a severe concern. In the early stages of resistances, only a small number of genes were identified, mainly TEM-1, TEM-2, and SHV-1 (Abdallah *et al.,* 2015; Paterson and Bonomo, 2005). There are more than 450 different TEM, SHV, and CTX-M enzyme variations that are produced by bacteria that host ESBL (Smet *et al.,* 2010). The goal of the current research had been to detect the ESBL isolates from various clinical isolates and to find the antibiotic resistant genes associated with them.

Materials and Methods

Collection of isolates and ESBL identification

The research was conducted in the Department of Veterinary Microbiology, FVSc & AH., SKUAST-K, and the Department of Microbiology, SMSR, Sharda University from January 2021 to December 2022, A total of 210 isolates of the *E. coli* have been gathered through the various sites in the Noida, Uttar Pradesh, and Kashmir valley regions. These isolates were from patients who may have had *E. coli* infections. Phenotypic identification was done by using ESBL chromogenic agar, Double disc synergy test, E-test, and Disk diffusion test. Presumptive isolates found to be positive in phenotypic tests underwent genotypic detection by multiplex PCR amplification utilizing specific primers set (Fang *et al.,* 2004; Monstein *et al.,* 2007; Boyd *et al.,* 2004).

Extraction of bacterial DNA

Utilizing a DNA extraction kit that is available commercially, the DNA was isolated from the bacterial lysate (Wizard Genomic DNA purification kit, Promega). This helps us to achieve the pure DNA without any PCR inhibitors. The DNA extracted was subjected to O.D check before using them as a template in the PCR reaction. A ratio of 260/280 in the range of 1.6-1.8 was appropriate to consider.

Molecular detection of *tetA, tetB, Sul1, Sul2, Dfrla, AadA*

Multi drug resistance ESBL positive isolates were tested for the presence of tetracycline (*tetA, tetB),* Sulphonamide *(Sul1, Sul2),* Streptomycin *(Dfrla),* Trimethoprim *(AadA)* genes by PCR. Using the Go-green Master mix, all of the PCR experiments in this investigation were carried out in a 25µl reaction volume (Promega). DNA extracted from above mentioned method was used as template subjected to PCR amplification for the detection of antibiotic genes utilizing a set of primers as mentioned in Table-1. The cyclic conditions are mentioned in Table-2.

Electrophoresis and Documentation

A 500 ml Erlenmeyer flask has been utilized to heat the necessary agarose quantity (Sigma Aldrich, St. Louis, USA) with 50 ml of a 1X tris acetate EDTA (TAE) (appendix) buffer. This resulted in an agarose gel that had a weight-to-volume ratio of 1.5 percent. After the flask had been chilled to 60 degrees Celsius, ethidium bromide has been poured until the final concentration read 0.5 µg per ml. After allowing the heated agarose solution to totally set at room temperature for 30 mins, the solution was poured into a plastic holder equipped with an appropriate comb (with one mm wells). Following the removal of the comb, the gel was installed on an electrophoresis tank (manufactured by Amersham Pharmacia Biotech, UK), and the electrophoresis tank was then filled with 1X TAE buffer. On the submerged gel, individual PCR samples were placed into wells of their own. In addition, the standard molecular weight (100 bp) marker that was supplied by promega was injected into one well. A voltage ranging from 1 to 5V/cm was applied across the gel till the yellow dye of the master mix migrated to the correct distance. After removing the gel, it was recorded using ultraviolet light,

and photographs were taken using the Gel Documentation System (Ultra-lumInc.Imaging System, UVP, UK).

Results and Discussion

ESBL Screening of *E.coli*

The initial screening test of ESBL production by DDST and the phenotypic confirmation test revealed that 158(or 75%) out of the 210 *E. coli* isolates were positive. Multiplex PCR was performed on all 158 phenotypically verified E. coli isolates using bacterial lysate as the template. Out of 158 ESBL isolates only 124 (78.48%) isolates carried the gene/s screened for blaTEM, blaCTX-M and blaSHV genes. Isolates carrying blaSHV gene produced an amplicon of 237 bp, those carrying blaTEM showed an amplicon of 445 bp. The blaCTX-M gene produced an amplicon of 593bp as depicted in Fig-1

Molecular detection of *tetA, tetB, Sul1, Sul2, Dfrla, AadA*

All the 124 isolates which were declared as multi drug resistance ESBL positive isolates, were tested for the presence of *tetA, tetB, Sul1, Sul2, Dfrla, AadA* genes. Singlelex PCR assay for each targeted gene using specific primers were performed. Antimicrobial gene profile revealed *tetA* present in 53.22% of isolates whereas *tetB* is present in 32.24% isolates.

Similarly *Sul1* and *Sul2* is present in *8.1%* and *16.52%* respectively. *tetA* and *tetB* produced an amplicon size of 937 and 659bp respectively as depicted in Fig-2 whereas the *Sul1* and *Sul2* produced an amplicon size of 789 and 722 respectively as depicted in Fig-3&4. None of the isolates showed the presence of *Dfrla* and *AadA* genes

The current study looks on the *Enterobacteriaceae* that produce ESBLs in two tertiary care hospitals in India. The distribution of *Enterobacteriaceae* that produce ESBL varies worldwide. Multiple drug-resistant *Enterobacteriaceae* have been found to be highly prevalent in the majority of nations where patients are receiving antibiotic treatment (Oberoi *et al.,* 2013).

However, lower rates were found in North America and Europe previously (Oberoi *et al.,* 2013; Cantón and Bryan, 2012) and high rates in Asia (Peirano and Pitout, 2019), South America (Jean and Hsueh, 2011; Bonelli *et al.,* 2014), and Africa (Leopold *et al.,* 2014). The factors responsible for the spread are encouraged by indiscriminate antimicrobial use without a prescription, use of counterfeit drugs, poor hygiene, a high prevalence of infectious diseases, and lack of diagnostic tools for detecting antibiotic resistance (Sonda *et al.,* 2016; Chong *et al.,* 2018). By understanding the mechanisms behind ESBL-producing Enterobacteriaceae and their association with MDR phenotypes, we can develop more effective strategies to combat these increasingly complex pathogens and safeguard public health. “The highest tetracycline resistance phenotypes observed in the *E. coli* isolates were linked to the presence of the *tetA* gene (53.22%) and considered to be the gene commonly identified followed by *tetB* (32.24%) in the *E. coli* isolates. They are among the most widespread *tet* genes found in Enterobacteriaceae and their occurrence was within the range reported by other investigators (Lanz *et al.,* 2003).

Because *sul1* is usually associated with transposons and integrons as part of the 3′ conserved structure, it was commonly identified in our *E. coli* isolates in agreement with earlier studies that reported *sul1* and *sul2* to be common among bacteria from the Enterobacteriaceae family (Antunes *et al.,* 2003). We confirmed the predominance of gene cassettes conferring resistance to streptomycin and to spectinomycin (aadA), trimethoprim (dfrA). The persistence of these genes, which have been reported worldwide in isolates from different origins, might be associated with the extensive use of streptomycin/spectinomycin, trimethoprimes, sulfonamides, and other antibiotics in food-producing animals”.

Based on the Phenotypic test results like E-test, double disk synergy test, and phenotypic confirmatory test (ESBL Chromogenic agar), we conclude that the isolates in this investigation are phenotypically presumptive positive ESBL *E. coli.*

Upon molecular analysis, 124 isolates tested positive for the genes *bla TEM, blaSHV*, and *blaCTX-M*, which are primarily expressed by isolates that are MDR ESBL. Therefore, one of the reasons for the development of AMR is the indiscriminate use of antibiotics in the food industry.

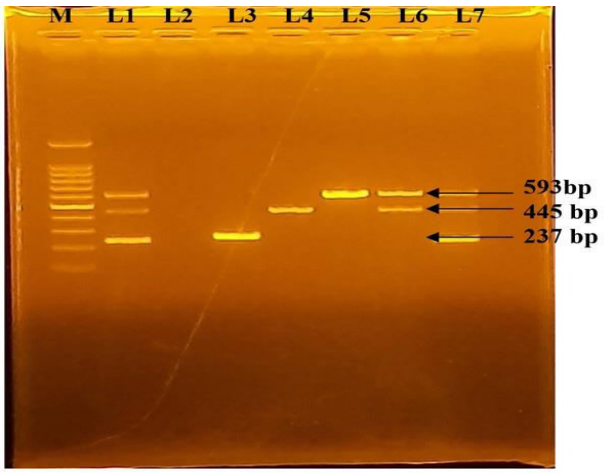
Table.1 Targeted genes for PCR amplification - *tetA, tetB, Sul1, Sul2, Dfrla, AadA*

Primer	Target gene	Amplicon size (bp)	References
TetA F *TetA* R	tetA	937	Guardabassi *et al*., (2000)
tetB F *tetB* R	tetB	659	Fonseca *et al*., (2006)
Sul1 F *Sul1* R	*Sul*1	789	Mazel *et al*., (2000)
Sul2 F *Sul2* R	*Sul*2	722	Maynard *et al*., (2003)
AadA F *AadA* A	*AadA*	282	Madsen *et al*., (2000)
Dfrla F *Dfrla* R	*Dfrla*	474	Navia, (2003)

Table.2 Cyclic conditions for PCR amplification of *tetA, tetB, Sul1, Sul2, Dfrla, AadA*

"Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension"
tetA	95°C for 5 min	95°C for 30s	52°C for 30s	72°C for 45s	72°C for 7 mins
tetB	95°C for 5 min	95°C for 30s	52°C for 30s	72°C for 20s	72°C for 10 mins
Sul1	94°C for 5 min	94°C for 30s	59°C for 30s	72°C for 60s	72°C for 8 mins
Sul2	94°C for 5 min	94°C for 30s	59°C for 30s	72°C for 90s	72°C for 7 mins
AadA	94°C for 5 min	94°C for 30s	50°C for 30s	72°C for 90s	72°C for 7 mins
Dfrla	94°C for 5 min	94°C for 30s	54°C for 30s	72°C for 60s.	72°C for 7 mins

Figure.1 Representative bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes profile of phenotypically positive isolates using multiplex polymerase chain reaction (m-PCR):-



Lane M: 100 bp DNA Ladder; Lane 1: Positive control for bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes; Lane 2: Negative control Lane 3: bla_{SHV} positive; Lane 4: bla_{TEM} positive; Lane 5: bla_{CTX-M} positive; Lane 6: bla_{TEM} and bla_{CTX-M} positive and Lane 7: bla_{SHV}, bla_{TEM} and bla_{CTX-M} positive

Figure.2 PCR detection of *tetA gene*:- M: 100 bp DNA Ladder; Lane 2 & 3 Positive for *tetA* gene; Lane 4: Positive control

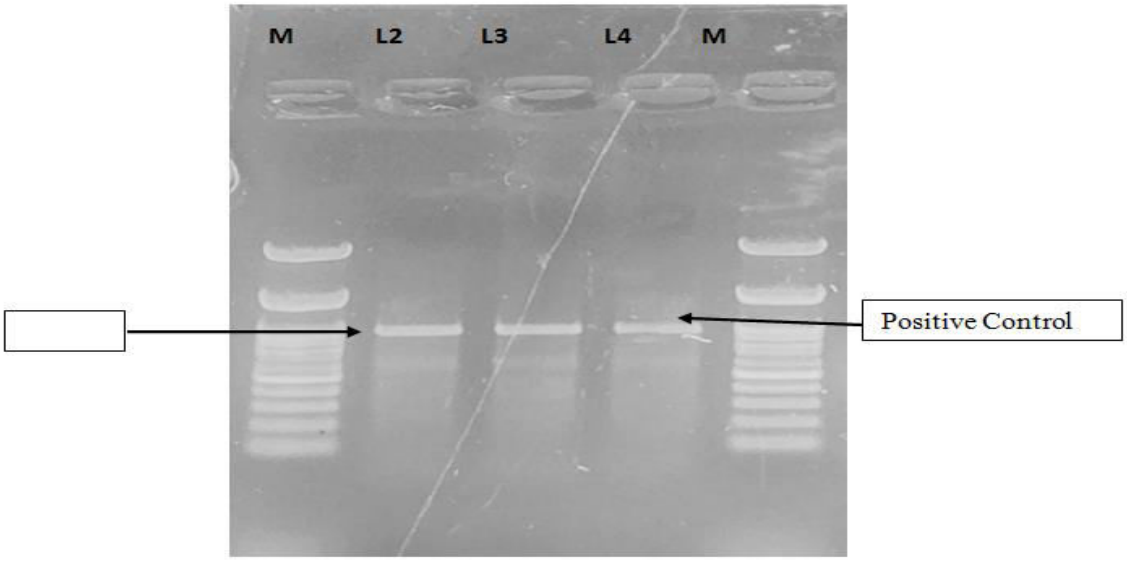


Figure.3 PCR detection of *Sul1 gene*:- Lane L1 to L5: Positive for *Sul1* gene M: 100 bp DNA Ladder; Lane 6: Positive control

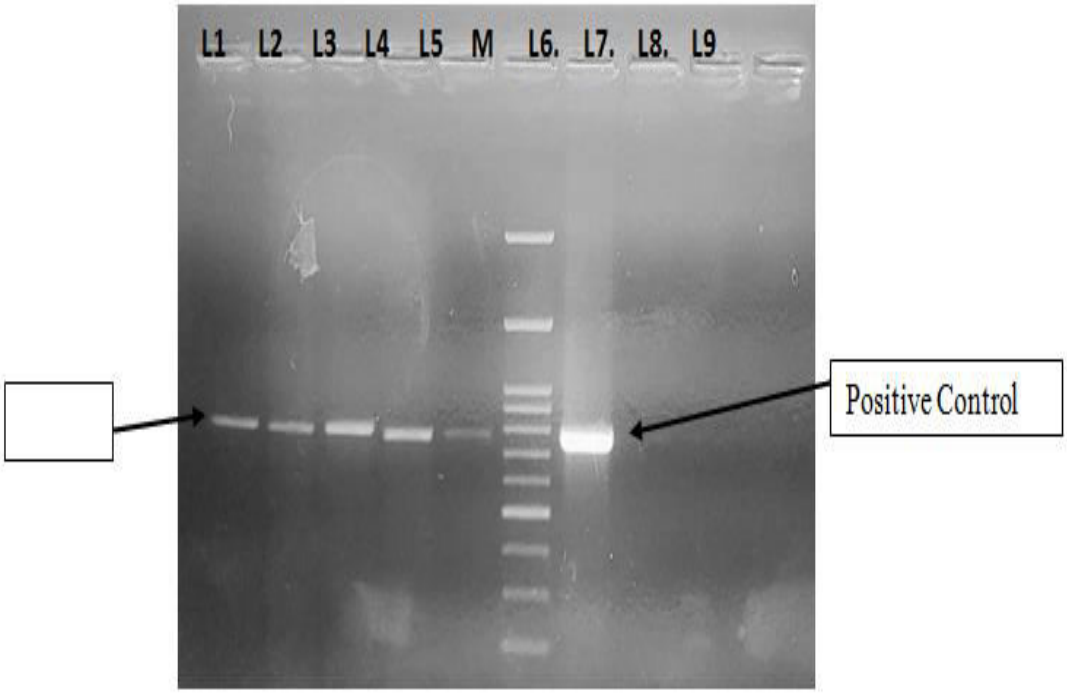
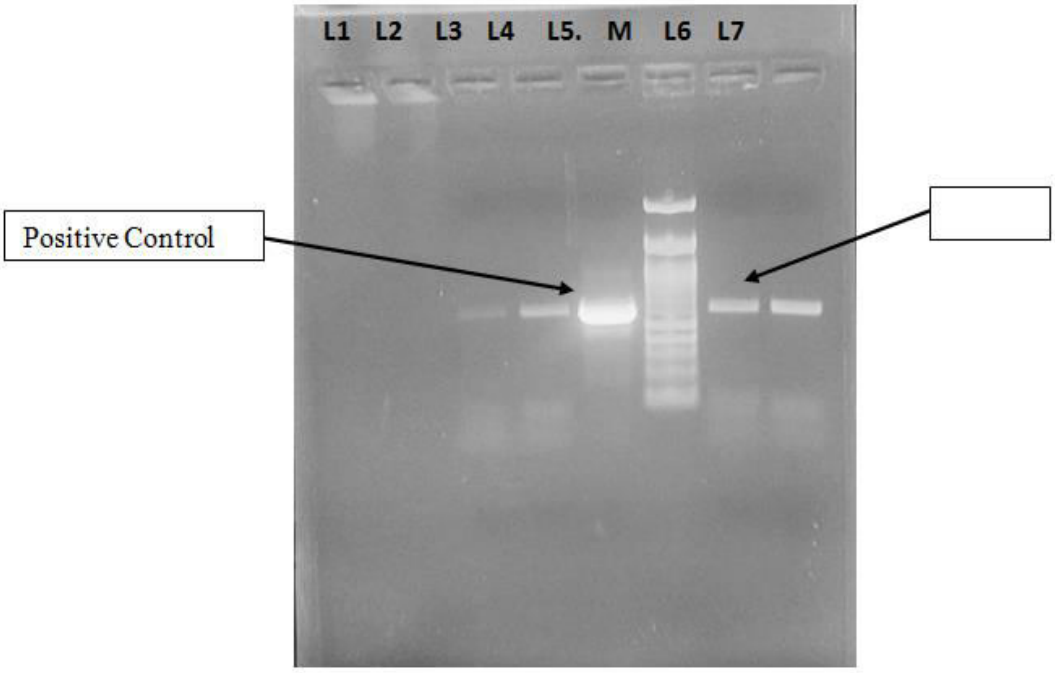


Figure.4 PCR detection of *Sul2 gene*:- Lane L3, L4, L6 & L7 Positive for *Sul2* gene M: 100 bp DNA Ladder; Lane 5: Positive control



To stop the spread of ARGs among humans and preserve the cultural practices of the pastoral communities, efforts should be made to develop medical and husbandry practices that will increase the availability of antibiotics while combating any medical health issue. Co-carriages were also observed in the ESBL isolates.

These genes are persistent, and reports of them in isolates from various origins around the world suggest that this is related to the widespread use of antibiotics in animals that produce food, such as trimethoprimes, sulfonamides, and streptomycin/spectinomycin.

Authors' Contribution

HAS, DKK, MAB conceptualized the work and conducted research. First author HAS also written the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest All authors declare there is not any conflict of interest or any affiliation or involvement in any organization whether it is academic, commercial, financial, personal and professionally.

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