IJBAHR

UNRAVELING THE ENIGMA OF ESBL: MOLECULAR DETECTION IN CLINICAL ISOLATES FROM KARACHI, PAKISTAN

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Abstract: ESBLs represent a plasmid-encoded group of diverse, complex and rapidly developing enzymes which challenge healthcare providers when treating hospital and community patients. These enzymes are produced by Gram-negative bacteria and provide resistance against third-generation cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone) and monobactams (e.g., aztreonam) and a wide spectrum of beta-lactam antibiotics but are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. The present study aims to detect ESBL gene in clinical isolates of *E.coli* from tertiary care hospitals. Cultures were collected, isolated and identified as *E.coli* using standard microbiological techniques. Among 80 isolates, 72 were CTXM1 positive and TEMF gene was positive in 51 isolates.

Key words: ESBL, *E.coli*, CTXM1, TEMF, nosocomial infection

INTRODUCTION

In Pakistan's medical setting, the existence of ESBL bacteria is a serious issue. Mutations that enhanced the hydrolysis of cefotaxime, ceftazidime, and ceftriaxone resulted from the evolutionary path from traditional beta-lactamases such as TEM-1, TEM-2, and SHV1 (Paterson & Bonomo, 2005).The inhibitory effect on beta-lactamase occurs through Ambler class A and class D beta-lactamases and these enzymes are blocked by clavulanic acid, sulbactam, or tazobactam (Paterson & Bonomo, 2005). AmpC is another beta-lactamase class that breaks down beta-lactams even though it shows no response to clavulanic acid treatment which differentiates it from ESBLs (Jacoby, 2009). The functional mechanism of ESBLs functions through enzyme activity that destroys the beta-lactam ring structure of vulnerable antibiotics thus making them useless. The main enzymatic component of ESBLs contains a serine residue which functions by nucleophilically attacking the beta-lactam ring amide bond for hydrolytic ring cleavage (Shaikh *et al*., 2015). The bacterial survival and multiplication in antibiotic zones result from beta-lactam antibiotic hydrolysis through PBP blockage performed by ESBLs (Bradford, 2001). Since the use of antibiotics puts bacteria under evolutionary pressure, ESBLs developed naturally through mutation. Scientists discovered TEM-1 beta-lactamase, which worked against penicillins and first-generation cephalosporins, in Escherichia coli bacteria in the 1960s (Dashti and Mahrez, 2025). When third-generation cephalosporins became accessible in the 1980s, bacteria with TEM-1 or SHV-1 mutations evolved the enzyme necessary to degrade the medications (Bradford, 2001).Because CTX-M-type ESBLs were initially discovered in *E.coli* and swiftly spread to *Klebsiella pneumoniae*, *Enterobacter cloacae*, and other Enterobacterales species, ESBL epidemiology underwent a significant development in the 1990s (Bonnet, 2004). Before being utilised to horizontally transfer these genes between various clinical pathogens, CTX-M beta-lactamases originated from Kluyvera spp., which are naturally present in the environment (Bevan et al., 2017). The CTX-M-type ESBLs are the most

common ESBLs in the world, and the two main variations are CTX-M-14 and CTX-M-15 (Peirano & Pitout, 2019). Serious bacterial infections have been caused by ESBL-producing bacteria, which have proliferated in nosocomial (healthcare) and public settings. Although researchers have confirmed that ESBLs can also be found in non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, they are mainly found in *Enterobacterales* bacteria (Peirano & Pitout, 2019). In Pakistan's medical setting, the existence of ESBL bacteria is a serious issue. A pooled prevalence rate of 40% was shown by Azzam *et al*.

(2024) in all of Pakistan's districts. Research revealed that CTX-M-1, TEM-1, and CTX-M-15 are the predominant genotypes of uropathogenic *E. coli* isolates from Faisalabad, accounting for 48% of ESBL enzyme production (Ehsan *et al.,* 2023). Healthcare workers became more reliant on carbapenem drugs after another study conducted in hospitals in Lahore found that 70% of samples contained ESBL-producing isolates of K. pneumoniae (Shah *et al*. 2003).).

MATERIALS AND METHODS

Culture Collection

Clinical bacterial cultures (plate and broth) were gathered from various diagnostic labs of Pakistani tertiary care hospitals in Karachi. Standard microbiological methods were used to isolate and identify the cultures (Whitman *et al.,* 2012). For the experiment, the cultures were then further preserved on slants and glycerol stocks.

DNA Extraction

The Boiling Method was used to extract the samples' DNA (Omer and Dablool, 2017). Sterile Eppendorf tubes were filled with 0.5 ml of injection water. A sterile wire loop was used to transfer the freshly isolated colony from the nutrient agar plate, and it was thoroughly mixed by vortexing. For ten minutes, the cell suspension was centrifuged at 10,000 rpm. The pellet was vortexed after being resuspended in 0.5 ml of water. For fifteen minutes, the eppendorf tubes were maintained at 100°C in a shaking water bath. The supernatant was moved to a fresh autoclaved eppendorf tube and stored at -20°C for later use after centrifuging at 10,000 rpm for 10 minutes.

DNA Quantification

The extracted DNA was quantified using a NanoDrop Spectrophotometer. On the NanoDrop lens, 3.0 µL of the sample was placed. To determine the purity of the DNA, absorbance values were measured at 260 and 280 nm. To identify protein or RNA contamination, the A260/A280 ratio was computed.

Polymerase Chain Reaction for ESBL Genes

The PCR assay was used to find the ESBL genes (CTXM-1 and TEMF). Primers were synthesized from Molecular Biology and extracted from the literature (Table 1).

Table 1: Sequences of the primers used in this study

Primers	Gene	Sequence
Forward	CTXM_F	ACCGCCGATAATTCGCAGAT
Reverse	CTXM_R	GATATCGTTGGTGGTGCCATAA
Forward	TEM_F	TTGGGTGCACGGCTGGGTTA
Reverse	TEM_R	TAATTGGTTGCCGGGAAGCTA

The PCR reaction mixture utilised in this investigation included forward and reverse primers (CTX-M1 & TEMF separately), as well as 12.5 µL of master mix. 6.7 µL of nuclease-free water, 1.0 µL each, and a DNA template supplemented with 5.0µL. The following program was used to perform PCR.

Table 2: PCR program used in this study

Step	Time Duration	Temperature
Initial Denaturation	7 minutes	95°C
Denaturation	45 sec	95°C
Annealing	40 seconds	60°C
Elongation	90 seconds	72°C
Final Extension	10 minutes	72°C
Total amplification cycles: 40		

Gel Electrophoresis for PCR Product Analysis

The PCR products were evaluated using Gel Preparation (2.0 % Agarose gel). 50 mL of 1X TAE buffer was heated in a microwave to dissolve 1 g of agarose. Ethidium bromide (final concentration of 0.5 µg/mL) was added and thoroughly mixed once the mixture had cooled to about 50°C. Using a comb, the solution was transferred into a gel casting tray and left for half an hour to solidify. Gel loading dye (1.0 µl) was combined with 5.0 µl of the PCR product. A 100 bp DNA ladder was used as a molecular weight marker. Electrophoresis was performed at 100 V for 30–40 minutes. A UV transilluminator was used to view the gel. The size of the PCR products was ascertained by comparing the bands with the DNA ladder.

RESULTS

Culture Isolation and Preservation

A total of (80) bacterial isolates were obtained from both the provided plate and slants cultures. Individual colonies were obtained after streaking on Nutrient agar followed by a 24-hour incubation period.

Culture Identification

The isolates were subjected to Gram staining. Gram staining and biochemical analysis indicated that the isolated cultures were *Escherichia coli*.

DNA Extraction and Quantification

DNA was successfully extracted from each isolate using the boiling method. The extracted DNA was quantified using a Nanodrop spectrophotometer.

PCR Amplification of ESBL Genes (CTXM1 and TEMF) and Gel Electrophoresis Product Analysis

Gene-specific primers that target the CTX-M1 β-lactamase gene were used for PCR amplification, A 2.0% agarose gel stained with ethidium bromide was used to analyze the PCR products, which were then shown under a UV lamp (Figure 1). The CTX-M1 gene was present in every tested isolate, as evidenced by the distinct bands in every sample that matched the predicted size of 588 bp. DNA samples were subjected to PCR amplification with the TEMF β-lactamase gene as the target. Following ethidium bromide staining, the amplified products were resolved on a 2% agarose gel and observed under a UV lamp (Figure 2). The molecular size marker was a 100 bp DNA ladder. The presence of the TEMF gene in these isolates was demonstrated by the

distinct bands of about 445 bp that were seen in Lanes 12, 13, 14, 15, 16, and 17. Samples 11, 18, 19, 20, and 21 showed no amplification, indicating that the gene was not present in these samples. The size of the amplicons was estimated using a 100 bp molecular weight marker.

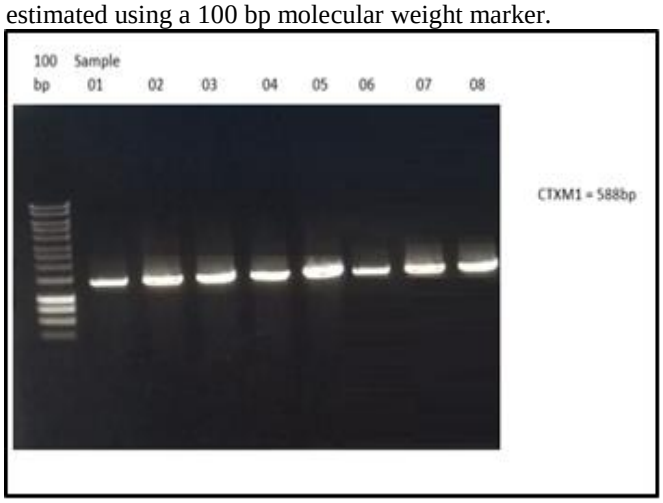

Fig1:Agarose gel electrophoresis showing PCR amplification of the CTX-M1 gene (~588 bp) from DNA samples. A 100 bp DNA ladder was used as a molecular marker (Lane 1). Lanes 02 to 08 represent clinical isolates screened for the presence of CTX-M1.

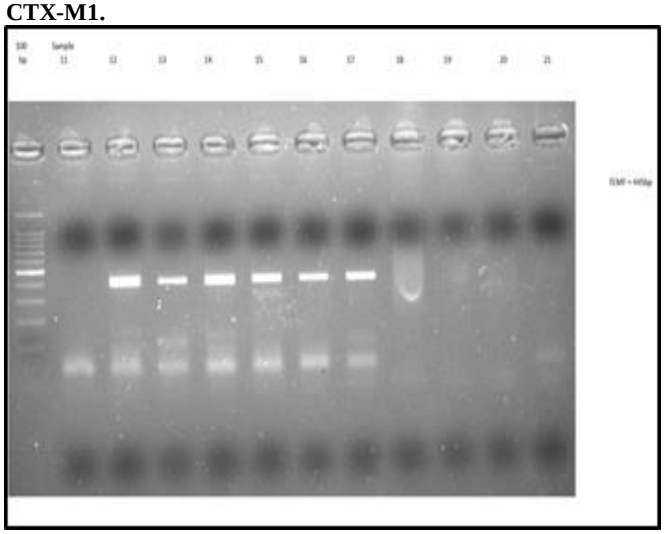

Fig 2: Agarose gel showing PCR products (~445 bp) of the TEMF gene from samples 11–21. Positive amplification is observed in samples Lane 12 to 17 whereas Lane 1 represents: 100 bp DNA ladder.

DISCUSSION

Escherichia coli that produces extended-spectrum β-lactamase (ESBL) is a serious threat to global health, particularly in low and middle-income nations where infection control and antibiotic stewardship are frequently insufficient (Dashti and Mahrez, 2025). The study aimed to isolate and molecularly characterise ESBL-producing *E.coli* from clinical isolates acquired from tertiary healthcare centres in Karachi, with a focus on the PCR detection of blaCTX-M and blaTEM genes. Among 22 isolates, 14 were PCR-proven ESBL producers using primers that targeted the blaCTX-M and blaTEM gene families. Finally, the most common gene type was CTX-M-1 type as the global ESBL resistance pattern shifted to favour the CTX-M enzymes over the already older TEM and SHV ones (Bonnet, 2004; Bevan *et al*., 2017). At the same time, even among isolates, notable rates of TEM-type ESBLs were noted. The TEM-1 enzyme and its near derivatives hydrolyse a broad range of β-lactam antibiotics, making it one of the earliest ESBLs to be identified (Bradford, 2001). The occurrence of blaTEM in clinical isolates indicates the continuous horizontal gene transfer and the related co-selection determinants of multidrug resistance, even though CTX-Ms surpassed it as the most common globally (Shaikh *et al*., 2015). A sign of horizontal gene transfer using plasmids carried out by transposons and integrons is the presence of multiple ESBL genes in a single isolate (Bevan *et al*., 2017). Because of its genetic flexibility, *E. coli* is more adaptable and resistant, which allows it to survive in a variety of settings, including ones with little to no antibiotic exposure and those with high or significant antibiotic exposure. The efficiency of β-lactam/β-lactamase inhibitor combinations, like piperacillin-tazobactam, which are commonly used as second-line therapy, may also be impacted by this co-carriage of ESBL genes (Rawat & Nair, 2010; Bush & Bradford, 2016). There are important therapeutic ramifications when CTX-M and TEM-type ESBLs coexist. Once believed to be dependable for treating *E. coli* infections, third-generation cephalosporins are currently losing their effectiveness. Because of this, carbapenems are usually used as a last resort; however, excessive use of these antibiotics has resulted in the development of Enterobacteriaceae that are resistant to carbapenems (CRE) (O Nell, 2016; WHO, 2020). In these circumstances, physicians are compelled to use the toxic substitute, colistin, which has a risk of nephrotoxicity and treatment failure (Evans & Amyes, 2014; Poole, 2005).

CONCLUSIONS

In addition to determining the molecular identities of the genes blaCTX-M1 and blaTEM, the study aimed to determine the frequency of *E. coli* that produced the Extended-Spectrum Beta-lactamase (ESBL) enzyme, which was isolated in Karachi's tertiary healthcare hospitals. The results show a very high frequency of the CTX-M1 gene, which is in line with global patterns showing that the CTX-M enzyme is the main source of resistance in *E. coli*, replacing the once common ESBLs (like TEM and SHV). Improved infection controls, antibiotic-specific policies, and more effective antimicrobial stewardship in hospital settings are necessary, as evidenced by the high prevalence of ESBL-producing *E. coli* in this study. These bacteria give doctors fewer treatment options, raise healthcare costs, and decrease the effectiveness of widely used antibiotics like third-generation cephalosporins. Because of the clinical significance of the results, a comprehensive policy of routinely monitoring resistance genes, enhancing the diagnostic capabilities of microbiological laboratories, and

planning educational campaigns that increase awareness of the prudent use of antibiotics are all essential to combating this challenging clinical problem. Hospital antibiograms that include molecular data can also direct a customised empirical treatment strategy

In conclusion, the study's findings provide important molecular-level proof of the ESBL gene's frequency in clinical *E. Coli* isolates from Pakistan and urge prompt action to stop the spread of these resistant pathogens in medical environments.

Conflict of interest

Authors declare no conflict of interest.

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Submitted on	***30-09-2025***
Revised on	***08-10-2025***
Accepted on	***12-10-2025***

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