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Research Article

ANTIBACTERIAL POTENTIAL OF L-TRYPTOPHAN ON EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* STRAINS: A PATHOLOGICAL PROMISING APPROACH

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ABSTRACT

Objective: The main aim of this study was to determine the extended-spectrum beta-lactamase (ESBL) production among 83 isolates of *Escherichia coli* as well as the antibacterial effect of a novel compound, L-Tryptophan Schiff base, on 10 different ESBL positive strains of *E. coli.*

Methods: Phenotypic ESBL activity in *E. coli* was confirmed by combined disc diffusion test according to clinical laboratory standard institute guidelines. Phenotypically positive ESBL clinical isolates were selected for molecular screening for synchronized detection of $bla_{CTX-M-15}$ gene. Antibacterial activity of L-Tryptophan Schiff base was evaluated against ESBL positive isolates. The effect of L-Tryptophan Schiff base on a polymerase chain reaction (PCR) amplified a product of CTX-M-15 gene was also evaluated.

Results: Antibiotic susceptibility screening showed resistance of ESBL positive isolates in the range of 18–96%. Disc diffusion test for phenotypic ESBL detection revealed that 99% (83/83) of isolates showed resistance to third and fourth generation cephalosporins (ceftazidime, cefotaxime, and cefepime) including ampicillin. L-Tryptophan Schiff base showed antimicrobial effect on *E. coli*. Molecular analysis for ESBL detection showed that 60% of strains were positive for $bla_{CTX-M-15}$ gene. L-Tryptophan Schiff base also had downregulating effect on CTX-M-15 gene.

Conclusion: This study presented an approach toward finding a suitable drug to reduce the serious infections caused by ESBL positive microorganisms. Phenotypic as well as molecular characterization was performed to get knowledge about the overall behavior of ESBL positive isolates of *E. coli.* L-Tryptophan Schiff base showed good antibacterial properties against ESBL positive isolates and a downregulating effect on PCR amplified product of CTX-M-15.

Keywords: *Escherichia coli*, Extended-spectrum beta-lactamase, $bla_{CTX-M-15}$, L-Tryptophan Schiff base.

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INTRODUCTION

Beta-lactamases are bacterial enzymes which hydrolyze the beta-lactam ring, usually found in all the beta-lactam antibiotics, resulting in less effective compounds [1]. The two major Gram-negative bacteria which produce extended-spectrum beta-lactamases (ESBLs) are *Escherichia coli* and *Klebsiella* species along with other non-enteric organisms such as *Pseudomonas aeruginosa* and *Acinetobacter* species [2]. ESBLs are mainly encoded by three genes, namely bla_{TEM}, bla_{SHV}, and bla_{CTX-M} [3]. ESBL producing strains have been inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [4]. ESBLs do undergo continuous modification, and at present, there are more than 300 various ESBL variants [5].

ESBLs produced by *Escherichia coli* and *Klebsiella* species cause urinary tract infections, bacteremia, septicemia, ventilated associated pneumonia, intra-abdominal, skin, soft tissue, and bloody, and diarrhea which can sometimes progress to more dangerous infections similar to blood poisoning, which can be life-threatening [6].

The main motive behind this study was to evaluate the ESBL production of clinical strains of *E. coli* through phenotypic testing. Apart from this, minimum inhibitory concentration (MIC) of all the isolates was carried out using MIC paper strip. This study was further extended to determine the antibacterial effect of a novel compound, L-tryptophan Schiff base, against *E. coli* isolates which were followed by static biofilm assay. Finally, ESBL positive isolates were subjected to DNA isolation and polymerase chain reaction (PCR) amplification to study the effect of L-tryptophan Schiff base on CTX-M-15 gene.

L-Tryptophan Schiff base [(S)-2-(2, 4-dihydroxybenzaldehyde) amino)-3-(1H-indol-3-yl) propanoic acid] (Molecular weight; 324.11Da) was supplied by Mr. R. Jayaprakash, Department of Chemistry, BSA Crescent Institute of Science and Technology, Chennai, India. The confirmed molecule was freely soluble in methanol, dimethyl sulfoxide (DMSO), N, N-dimethylformamide, and water. Out of these three solvents, DMSO was the best solvent required in minimum quantities. pH of the compound was more stable in aqueous media. The compound contains azomethine group (-CH=N-), carboxylic acid group, and two hydroxyl groups, indole moiety and an asymmetric carbon atom which are active pharmacophores in medicinal field. The structure enzyme inhibition predicted value was 0.23 (Molinspiration). It showed four hydrogen bond donors [7]. The chemical structure of L-Tryptophan Schiff base is shown in Fig. 1.

METHODS

Ethical clearance

This study was permitted by the Institutional Ethics Committee of School of Life Sciences, B. S. Abdur Rahman Crescent Institute of Science and Technology (Ref. no. BSAU: REG-OFF: 2016/02SLS), Vandalur, Chennai.

Collection and maintenance of bacterial strains

A whole set of 83 different clinical isolates of *E. coli* were obtained with information on gender and age from the Department of Microbiology,

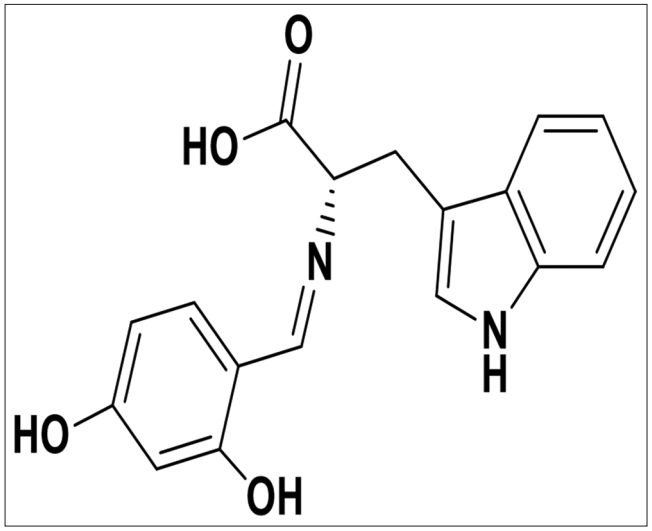

Fig. 1: Chemical structure of L-Tryptophan Schiff base

Tagore Medical College and Hospital, Chennai, India, within the time span of 6 months. The isolates included urine culture (n=65), blood culture (n=6), wound swab culture (n=2), pus culture (n=1), vaginal swab culture (n=1), and hand wash swab (n=8). The bacterial isolates were identified by following Bergey's manual of bacteriology which includes a morphological appearance of the colonies, staining methods, and biochemical properties [8]. The clinical isolates were maintained as glycerol stocks for further use.

Antibiotic susceptibility testing

The clinical strains were tested for antimicrobial susceptibility test by disc diffusion method [9]. In brief, each test isolate was seeded on Mueller Hinton Agar (MHA). After incubating overnight at 37°C, one colony from each isolate was inoculated in sterile peptone broth and the OD was adjusted to 0.5 McFarland standards. The suspension was swabbed on surface of MHA plate and left for 5–8 min. Required antibiotic discs were placed, and complete setup was incubated at 35±2°C for 18–24 h. The following antibiotics were used; amoxicillin (10 µg), aztreonam (10 µg), cefotetan (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefpodoxime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (5 µg), and meropenem (10 µg).

Phenotypic test for ESBL production

All the clinical strains showing the diameter of <26 mm for cefotaxime, <21 mm for ceftazidime, <18 mm for cefepime, and <19 mm for amoxicillin were selected for ESBL production through phenotypic analysis. Phenotypic ESBL confirmatory test was executed in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines [10] using quality control reference strain of *E. coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 700603). All the isolates were selected on the basis of initial screening. A combined disc diffusion test was done by placing a combination of antibiotic discs such as: Ceftazidime (CAZ 30 µg) versus ceftazidime/clavulanic acid (CAZ/CA 30/10 µg), cefotaxime (CTX 30 µg) versus cefotaxime/clavulanic acid (CTX/CA 30/10 µg), cefepime (CPM 30 µg) versus cefepime/clavulanic acid (CPM/CA 30/10 µg), and amoxicillin (20 µg) versus amoxicillin/clavulanic acid (AMC/CA 20/10 µg) on MHA plates on which inoculum was spread. The interpretation of test result was done by following CLSI guidelines and an approximate increase of ≥5 mm zone of inhibition for the disks containing CTX/CA, CAZ/CA, CPM/CA, and AMC/CA versus the comparative and alone CTX, CAZ, and AMC disc, was taken as ESBL positive. MIC of all ESBL producing isolates was evaluated using a unique MIC determination paper strip [11]. Three different MIC strips (HiMedia) coated with cefotaxime, cefepime, and ceftazidime in the range of 0.016–256 µg/ml were used on MHA. *E. coli* (ATCC 25922) was used as a reference strain.

Antibacterial effect of L-Tryptophan Schiff base

MIC and minimum bactericidal concentration (MBC) of L-tryptophan Schiff base

The MIC of L-Tryptophan Schiff base was performed through micro broth dilution technique following CLSI guidelines [10]. MIC was calculated as the minimum concentration of L-Tryptophan Schiff base that inhibited the bacterial growth. MBC was calculated by plating all MIC dilutions lacking observable turbidity. 10 µl aliquots from MIC dilution were plated on MHA plates and incubated at 37°C for 24 h. The MBC was considered to be the lowest concentration of L-Tryptophan Schiff base compound that completely reduced the growth of bacteria in 18–24 h.

Antimicrobial activity through well plate method

Antimicrobial activity of L-tryptophan Schiff base was evaluated against 10 ESBL positive strains of *E. coli* and one ATCC *E. coli* strain by agar diffusion test following CLSI guidelines [10]. 5 mg of L-tryptophan Schiff base was dissolved in 1 ml of 50 % DMSO. 6 mm wells were cut and 50 µl of compound was loaded in each well. 50% DMSO was used as a control and plates were incubated for 18–24 h at 37°C. The inhibition zone was determined by measuring the diameter of the clear zone around each well.

Static biofilm assay

The effect of L-Tryptophan Schiff base on biofilm formation by an ATCC strain of *E. coli* was perceived through the modified methods of Rina *et al*., 2014 [12]. Overnight *E. coli* cultures were diluted in fresh Brain heart infusion broth medium (1:100) in borosilicate glass tubes which contained the two-fold reducing concentrations of L-Tryptophan Schiff base only (7.5–60 µg/ml). Tubes with strain and no compound served as a positive control, while the tubes with only broth served as a negative control. All cultures were incubated for 24 h. After proper incubation, the medium was discarded and the tubes were washed with double distilled water followed by the addition of 0.1% crystal violet all tubes. After 15 min of standing, the additional dye was rinsed with phosphate buffered saline. The emergence of biofilm was confirmed by the continuation of a pinkish ring in the tubes.

Scanning electron microscopy (SEM)

ATCC strain of *E. coli* cultured and adjusted to 0.5 McFarland standard was treated with the MIC concentration of L-Tryptophan Schiff base for 45 min for SEM analysis [12]. Phosphate buffer without Ca^{+2} and Mg^{+2} was used to wash the cell pellets and the washing was repeated 3 times. The cells were then fixed for 1 h in 2.6% glutaraldehyde (pH 7.2) at room temperature. Samples after fixation were allowed to settle for 24 h at 4°C. A 100 µl sample was suspended on a glass grid covered with ε-poly-l-lysine for 1 h, followed by 1 h of fixation with 2% (w/v) osmium tetroxide, and dehydration with ethanol and Freon. The final step was performed through the coating of samples with carbon followed by visualization in a FEI Quanta FEG 200 SEM.

Molecular analysis

Preparation of DNA template

ESBL positive clinical isolates confirmed by phenotypic characterization were selected for molecular screening for synchronized detection of the $bla_{CTX\text{-}M\text{-}15}$ gene by PCR [13]. Overnight bacterial cultures (18–24 h) prepared on LB agar plates were used to prepare template DNA. Two single colonies of *E. coli* were transferred to 150 µl of double distilled water. The bacterial suspension was boiled at 95°C for 10 min for lysing the bacterial cells. This ultimate lysate cell was placed on ice box for 1 min and the pellet was discarded with centrifugation at 12000 rpm for 5 min. Ultimately, that supernatant was used as a template DNA for amplification [14].

PCR amplification of $bla_{CTX\text{-}M\text{-}15}$ genes

ESBL producing isolates were amplified along with $bla_{CTX\text{-}M\text{-}15}$ gene. The amplification was performed using Mastercycler nexus gradient

(Eppendorf, USA). The reaction mixtures comprised 5 µl of 2× redeye Master Mix (Amplicon III), 2 µl from 1M CTX-M-15 forward primer: 5'-CACGTCAATGGGACGATGT-3'and reverse primer:5'-GAAAGGCAATACCACCGGT-3'each and 3 µl from template DNA were used to obtained 410 bp amplicon. The final volume was 10 µl. The amplification reaction was carried out as the follows; initial denaturation at 94°C as 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing CTX-M-15 for 58°C, extension at 70°C for 1 min, and the final extension at 72°C for 10 min. The PCR product was examined through electrophoresis (1.5 % w/v agarose gel) at 60 V for 60 min. 1× Tris-acetate-EDTA buffer (1× TAE buffer), pH-7.6, 20 mM acetic acid, 1 mM EDTA, and 100 bp DNA ladder (GeneDireX) were used. Gel was stained using ethidium bromide and imaged under ChemiDoc (Bio-Rad).

DNA sequencing of $bla_{CTX-M-15}$

$bla_{CTX-M-15}$-producing *E. coli* strains were selected for gene sequencing. The PCR products were purified using spin column PCR products purification kit (Eurofins Genomics India Pvt. Ltd., India) according to manufacturing instructions. The sequencing of the beta-lactamase gene PCR product was carried out utilizing both forward and reverse primers. Primers for sequencing $bla_{CTX-M-15}$ product were same as PCR primers. The sequencing result was compared with the sequence of *E. coli* (accession no. 048935) on NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Pairwise alignment of sequence was done utilizing the software ClustalW.

Effect of L-Tryptophan Schiff base on PCR amplified product of CTX-M-15

Genomic DNA of a single ESBL positive isolate of *E. coli* (cefotaxime resistant) was subjected to PCR analysis and the effect of L-Tryptophan Schiff base was studied. This study was carried out to study the effect of L-Tryptophan on the CTX-M-15 gene. Briefly, an ESBL producing strain was kept as a control (no treatment); same ESBL strain was treated with L-Tryptophan as well as with the antibiotic to study the effect. An ATCC strain of *E. coli* was kept as a negative control. After amplification of the genomic DNA, the samples were run on an agarose gel to observe the band intensities.

RESULTS

Antibiotic susceptibility testing

Antibiotic susceptibility screening showed powerful resistance of ESBL positive isolates toward ampicillin (96%), cefpodoxime (95%), aztreonam (90%), gentamicin (84%), ciprofloxacin (81 %), levofloxacin (81%), cefotetan (51%), meropenem (53%), and imipenem (18%) individually (Fig. 2) revealing that these drugs might be problematic if used for ESBL infections except imipenem to which *E. coli* isolates showed less resistance [15].

Disc diffusion test revealed that out of the 83 *E. coli* clinical isolates, 99% (83/83) showed resistance to third and fourth generation cephalosporins (ceftazidime, cefotaxime, and cefepime) including ampicillin. CSLI phenotypic confirmatory test using combined disc diffusion method (cephalosporin/clavulanate combination discs) showed improved susceptibility of maximum number of *E. coli* isolates toward cefotaxime, ceftazidime, cefepime, and amoxicillin together with clavulanic acid, thus confirming the production of ESBLs. *E. coli* strains were considered as ESBL positive for a minimum of one of the positive confirmation tests by combination disc. The antibiotic combinations include: CAZ/CLA, CTX/CLA, CPM/CLA, and AMX/AMC. Phenotypic detection of ESBL production is shown in Fig. 3 MIC determination of ESBL positive *E. coli* strains to third and fourth generation cephalosporins revealed that all of them were in the range of 16 to >256 µg/ml. MIC of cefotaxime was in the range of 128 to >256 µg/ml, ceftazidime in the range of 128 to >256 µg/ml, cefepime in the range of 16 to >256 µg/ml, and amoxicillin in the range of 32 to >256 µg/ml, revealing that the MIC is not a dependable approach in detecting the ESBL positive isolates [16].

MIC and MBC of L-Tryptophan Schiff base

The primary step in performing the antimicrobial activity of L-Tryptophan Schiff base was the estimation of MIC and MBC against 10 ESBL positive *E. coli* clinical isolates. Total MIC and MBC were carried out in Mueller Hinton broth medium. Out of the 10 examined *E. coli* strains, 4 strains showed MIC at 7.5 µg/ml, 4 strains at 9.8 µg/ml, and 2 strains at 10.3 µg/ml. MBC was found to be higher than MIC, depicting the bactericidal properties of L-Tryptophan Schiff base against *E. coli*. MIC and MBC of a control *E. coli* strain (ATCC 25922) were found as 7.0 and 8.0 µg/ml, respectively (Table 1).

Antibacterial activity of L-Tryptophan Schiff base through well plate method

Antimicrobial effect of L-Tryptophan Schiff base on *E. coli* was carried on MHA media by agar diffusion method. The result was measured through the zones of inhibition determining the efficacy of the compound to inhibit the growth of bacteria. Zones were measured with the help of HiMedia zone scale. The zone of inhibition for all the 10 isolates of *E. coli* is summarized in Table 2. The results suggested that L-Tryptophan Schiff base is a good antibacterial agent and can be an alternative option for antibiotics against clinical *E. coli* strains.

Static biofilm assay

L-Tryptophan Schiff base was found to inhibit the biofilm formation in an ATCC strain of *E. coli*. Static biofilm was completely prevented even

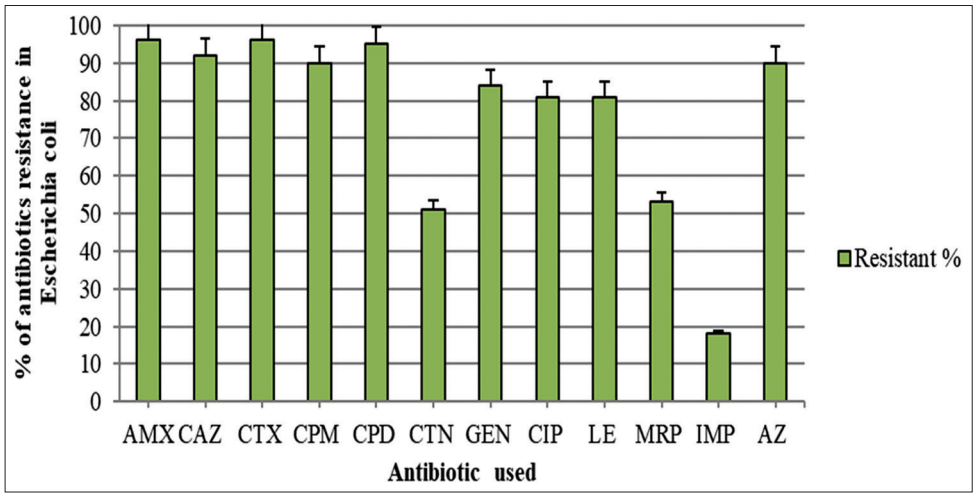

Fig. 2: Antibiotic screening by disc diffusion method

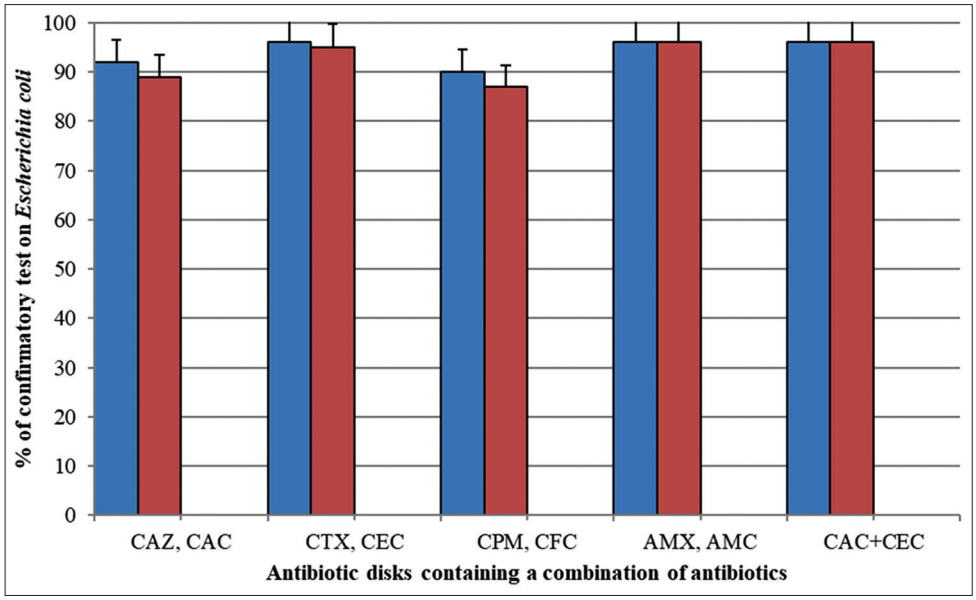

Fig. 3: Extended-spectrum beta-lactamase phenotypic confirmatory test by combined disk diffusion test

Table 1: MIC and MBC for L-Tryptophan Schiff base on clinical isolates of EC

EC isolate code	MIC (μg/ml)	MBC (μg/ml)
EC1	9.8±0.3	10.2±0.5
EC2	9.8±0.4	10.2±0.4
EC3	9.8±0.3	10.2±0.5
EC4	9.8±0.2	10.2±0.5
EC5	7.4±0.2	8.0±0.3
EC6	7.4±0.2	8.0±0.3
EC7	7.4±0.2	8.0±0.3
EC8	7.4±0.2	8.0±0.3
EC9	10.3±0.6	12.5±0.3
EC10	10.3±0.5	12.5±0.6
EC 11 (ATCC 25922)	7.0±0.3	8.0±0.2

EC: *Escherichia coli*, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

Table 2: Diameter of ZOI L-Tryptophan Schiff base against EC isolates

EC strain code	ZOI with L-Tryptophan (mm)	ZOI with DMSO (mm)
EC1	16±0.4	No zone
EC2	17±0.5	-
EC3	17±0.5	-
EC4	17±0.7	-
EC5	18±0.9	-
EC6	18±1.0	-
EC7	16±0.7	-
EC8	17±0.5	-
EC9	18±0.7	-
EC10	16±0.3	-
EC 25922 (ATCC strain)	18±0.0	-

EC: *Escherichia coli*, ZOI: Zone of inhibition, DMSO: Dimethyl sulfoxide

at the lower concentrations of L-Tryptophan Schiff base (lower than MIC) (Fig. 4 and Table 3). Biofilm production was higher in the positive control (without L-Tryptophan Schiff base).

SEM

The morphological changes in the cells were visualized through SEM analysis to get a knowledge about the biological overview of the bactericidal effect of L-Tryptophan Schiff base on ATCC strain of *E. coli* (Fig. 5a). The MIC concentration used to treat the cells was effective in distorting the shape of the cells as shown in Fig. 5b and c. The cells used for this study were cultured in LB broth and adjusted to 0.5 McFarland standard followed by the treatment with L-Tryptophan Schiff base.

Molecular analysis

Phenotypic ESBL-producing isolates were taken for molecular characterization using conventional PCR to target $bla_{CTX\text{-}M\text{-}15}$ gene and the amplified product was confirmed by gene sequencing. 60% of strains were found to be $bla_{CTX\text{-}M\text{-}15}$ positive (410 bp amplicon) out of 83 phenotypically characterized ESBL positive strains as shown in Fig. 6.

Gene sequencing analysis

The sequencing results of $bla_{CTX\text{-}M\text{-}15}$ after amplification are presented in Fig. 7. The sequencing results present a contrasting result of similarity, gaps and dissimilarities. Sequencing results were obtained by comparing the results with the sequence of *E. coli* (Accession no. 048935) in NCBI BLAST.

Effect of L-Tryptophan Schiff base on PCR amplified product

The effect of L-Tryptophan Schiff base on CTX-M-15 gene was evaluated on an ESBL producing strain of *E. coli.* The effect was studied as different band intensities after PCR amplification. Low band intensity was observed in the treated strain as compared to the control (without treatment) and the strain treated with antibiotic (cefotaxime), showing the downregulating effect of L-Tryptophan Schiff base on CTX-M-15 gene. The size of the amplified product was 410 bp (Fig. 8).

DISCUSSION

In the present society, pathogenic bacteria are a main reason behind severe diseases which make it very difficult to manage the patients inside hospitals. Microorganisms which are able to produce ESBLs have evolved as significant pathogens are very common and are often related to various epidemics. These microorganisms are resistant to a wide range of antibiotic formulations and hence pose a threat in the treatment of various diseases [17,18].

This particular study was performed for the determination of antibiotic susceptibility of phenotypically characterized ESBL positive isolates of *E. coli* by disc diffusion method revealing that ESBL positive isolates showed strong resistance toward a wide range of antibiotics such as ampicillin, cefpodoxime, aztreonam, gentamicin, ciprofloxacin, levofloxacin, cefotetan, and meropenem. Compared to these antibiotics,

Table 3: Biofilm inhibition by L-Tryptophan Schiff base

L-Tryptophan Schiff base concentration	Control (+ve)	Control (−ve)	7.5 µg/ml	15 µg/ml	30 µg/ml	60 µg/ml
Static biofilm formation	+	-	±	-	-	-

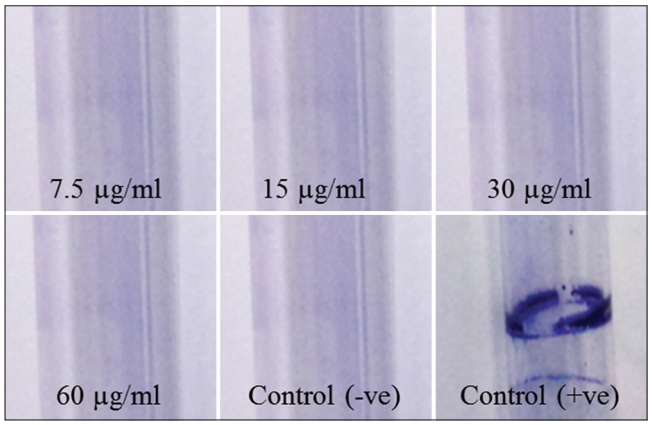

Fig. 4: Effect of L-Tryptophan on biofilm production by *Escherichia coli* strain (ATCC 25922)

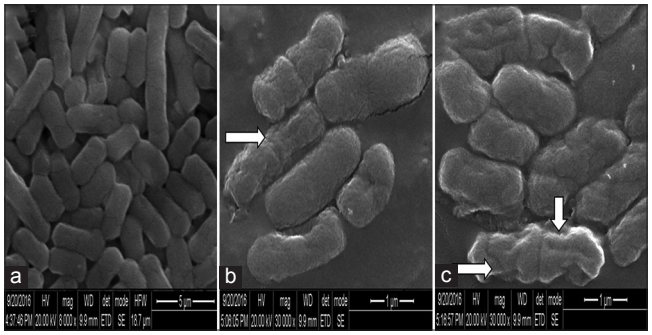

Fig. 5: Scanning electron microscopic images showing (a) the cells without treatment and (b and c) the effect of L-Tryptophan Schiff base on ATCC strain of *Escherichia coli*

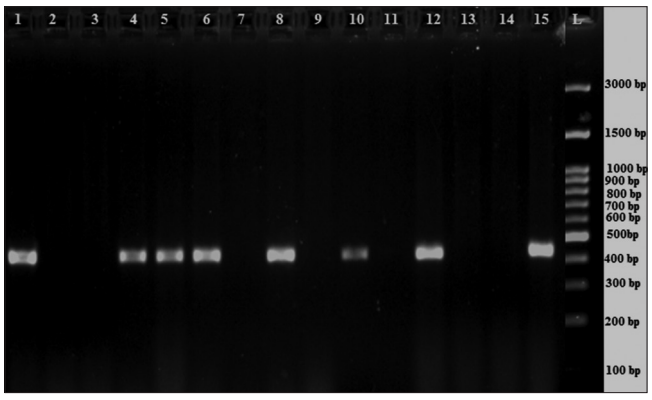

Fig. 6: Polymerase chain reaction amplification products of blaCTX-M-15 gene (Lane L: 100bp DNA ladder, Lane 1, 4, 6, 8, 10, 12, and 15: Isolates positive for blaCTX-M-15 gene, Lane 2, 3, 7, 11, 13, and 14: Isolates negative for blaCTX-M-15 gene.

all isolates showed less resistance toward imipenem and hence it can be concluded that imipenem could be a drug of choice for most of the isolates used in this study.

The phenotypic characterization for ESBL production was performed through combined disc diffusion test following CLSI guidelines. 99% of *E. coli* isolates showed resistance to the third and fourth generation cephalosporins. There are numerous other studies that have been carried out related to the percentage of *E. coli* isolates showing resistance toward cephalosporins. According to a study carried out by Sridhar *et al.* (2014), 61.4% of ESBL production was found in *E. coli* [14]. One of the studies carried out by Harwalkar *et al.* (2013) reported that 49% of the urinary *E. coli* strains showed a notable resistance toward one of the cephalosporins (Cefpodoxime, Ceftazidime, and Cefotaxime) [19].

This study further presented the detection of MIC's of ESBL producing *E. coli* isolates through MIC paper strip coated with cefotaxime. The results presented a range of MIC values between 16 and > 256 µg/ml which gave information about the higher range of resistance showed by ESBL positive *E. coli* isolates against third- and fourth-generation cephalosporins. ESBL producing microorganisms have restricted the worth of broad-spectrum cephalosporins in controlling the infections of these pathogens as there have been various reports of therapeutic catastrophes and higher death rates associated with broad-spectrum cephalosporins and hence the use of cephalosporins for treating grave and bloodstream related infections caused by ESBL producing microorganisms is not recommended [20].

This particular study was also performed to molecularly characterize the ESBL isolates through amplification using $bla_{CTX\text{-}M\text{-}15}$ gene. The PCR products were sequenced through pairwise sequence alignment. Based on different geographical reports, CTX-M-14 and CTX-M-15 are the major essential types of genes which are toxic and pose a threat to human as well as animal health [21]. Previous studies on CTX-M, TEM, and SHV type ESBLs have reported that these ESBL types are becoming more prevalent in India as well as all over the world. One more study about multiplex PCR for TEM, SHV, and CTX-M reported that 59.3% of *E. coli* harbors the CTX-M gene. CTX-M-15 ESBL has been found in *E. coli* isolates in Nigeria and Enterobacterial isolates in India [22]. These enzymes are increasing day by day and are being expressed by several groups of pathogenic bacteria especially the Enterobacteriaceae family, thereby compromising the activity of broad-spectrum drugs, hence causing a hindrance in treating the infected patients.

A novel compound, namely L-Tryptophan Schiff base was evaluated for its antibacterial activity against ESBL positive isolates of *E. coli.* MIC, MBC, and the antibacterial activity of L-Tryptophan Schiff base against ESBL positive isolates through well plate method showed positive results. Furthermore, this novel compound was able to inhibit the formation of biofilm in an uropathogenic strain of *E. coli.* A lot of studies have been put forward regarding the biofilm resistance of ESBL strains toward antibiotic formulations. Biofilm-associated microbes persist for long time in various atmospheres. Antibiotics resistance dramatically increases for the microorganisms with biofilm properties. Recently, a study on MDR strains of *E. coli* reported 92% biofilm production in Karnataka, India [23]. One more study from Coimbatore, India, has reported 60.2% biofilm production of *E. coli* ESBL producing strains [24]. Another *in vitro* study reported the uropathogenic *E. coli* biofilm production was highly resistant to antibiotics [25]. In this study, the stationary biofilm production by *E. coli* was inhibited by the MIC of L-Tryptophan Schiff base indicating the capacity of this compound to act as a potent growth and biofilm inhibitor. The effect of L-Tryptophan Schiff base also resulted in the downregulation of CTX-M-15, a gene which is most commonly found in the ESBL positive isolates and which provides an extra resistance toward antibiotics.

CONCLUSION

ESBL producing microorganisms pose a serious threat in the clinical practice because of their resistance to a wide range of antibiotic

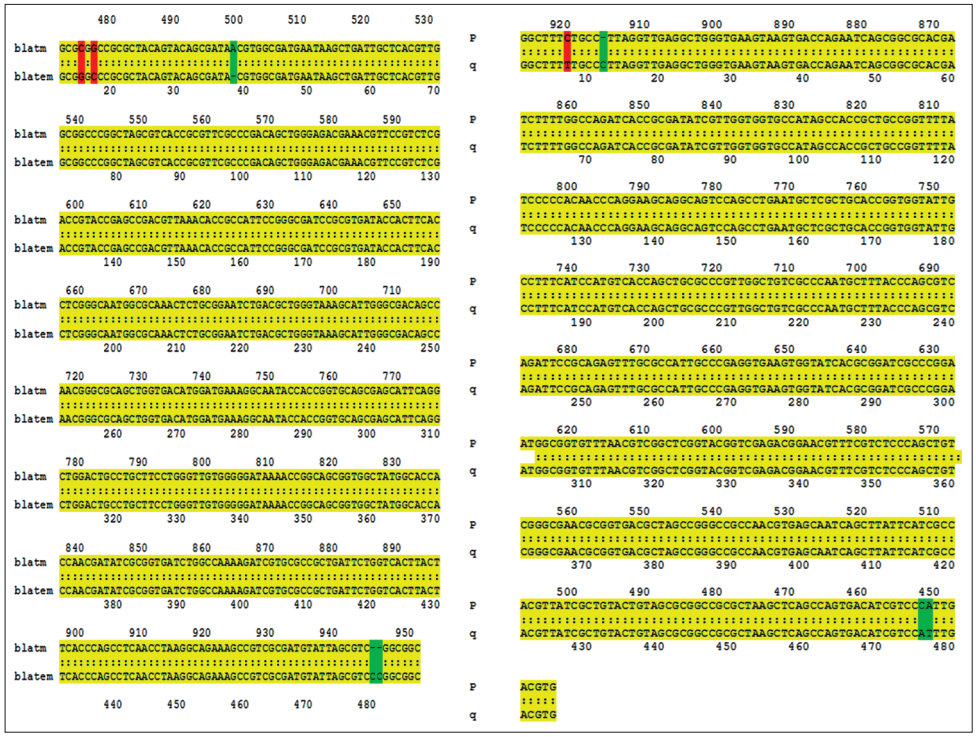

Fig. 7: The pairwise sequence alignment of the blaCTX-M-15 gene (yellow color highlighting their similarity, green color highlighting a gap, and red color highlighting dissimilarity of nucleotide)

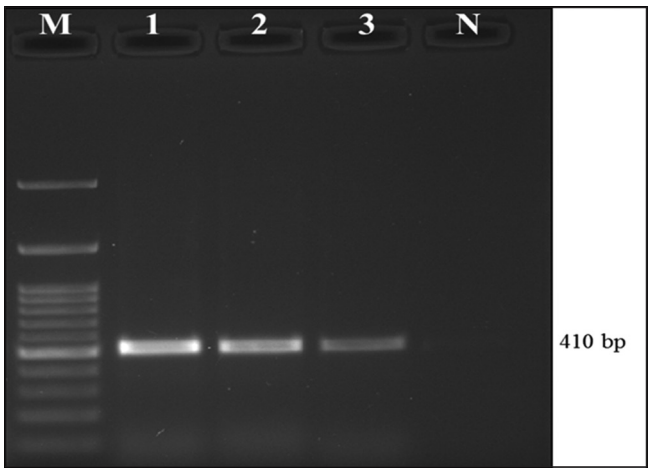

Fig. 8: Effect of L-Tryptophan Schiff base on CTX-M-15 gene (M = 100 base pair marker, Lane 1: Untreated, Lane 2: Treated with cefotaxime, and Lane 3: Treated with L-tryptophan Schiff base, N: Negative control)

formulations. This study presented an approach toward finding a suitable drug to reduce the serious infections caused by these microorganisms. Phenotypic as well as molecular characterization was performed to get knowledge about the overall behavior of ESBL positive isolates of *E. coli*. Gene sequencing was performed and the results were analyzed through pairwise alignment to find the similarities, dissimilarities and gaps by comparing the results already present in NCBI BLAST. A novel compound, L-Tryptophan Schiff base was assayed for its antibacterial properties against ESBL positive isolates, which showed the capacity of this novel compound as a good antibacterial agent. The effect of L-Tryptophan Schiff base on PCR amplified product of CTX-M-15 was also evaluated. In a nutshell, this particular study provided an insight on the behavior of ESBL positive isolates of *E. coli* in the presence of different antibiotic formulations as well as in the presence of a novel compound, L-Tryptophan Schiff base.

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AUTHOR'S CONTRIBUTION

The authors of this manuscript have contributed wholly or partly in conducting this work and preparing this manuscript.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

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