Original Article

Differential Expression of *bla*$_{CTX-M-33}$ with Vancomycin/Trimethoprim Combination in *Escherichia coli*-Producing Extended-Spectrum β-Lactamase Isolated from Intensive Care Unit-Acquired Urinary Tract Infection

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Abstract

Aim: The aim of this study was to develop combination approach for the treatment of *Escherichia coli*-producing extended-spectrum β-lactamases (ESBLs) isolated from intensive care unit (ICU)-acquired urinary tract infections (UTIs). **Materials and Methods:** The observational study was conducted between January 5, 2018- June 5, 2019 to isolate and detect *E. coli* from UTI patients admitted to ICUs in Shahid Rajaee hospital, Gachsaran, Iran. Morphological, biochemical and molecular methods were conducted to identify *E. coli* isolates. Phenotypic confirmation of *E. coli* producing ESBL was performed using ESBL disc diffusion test according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The combination index assay set-up was based on CLSI guidelines to investigate antibacterial susceptibilities to vancomycin alone and in combination with trimethoprim and interpreted with the fractional inhibitory concentration (FIC) index. Eventually, the expression levels of *bla*$_{CTX-M-33}$ gene were determined by real-time quantitative reverse transcription–polymerase chain reaction. **Results:** A total of 90 ICU-acquired UTIs occurred among 255 patients. The combination index assay results showed that vancomycin/trimethoprim combination would be reduced its minimal inhibitory concentration$_{90}$ value. The vancomycin/trimethoprim combination revealed partial synergistic and indifferent effects (FIC = 0.52–1.50) in the isolates of *E. coli*-producing ESBL. The results of gene expression analysis indicated that vancomycin/trimethoprim combination caused downregulation of *bla*$_{CTX-M-33}$ gene at negligible levels by 55.56–58.82-fold and stopped drug resistant. **Conclusion:** Vancomycin/trimethoprim combination may diminish resistance in the *E. coli*-producing ESBL isolated from ICU-acquired UTI patients.

Keywords: *Escherichia coli,* gene expression, therapeutics, trimethoprim, urinary tract infections, vancomycin

Introduction

The most important cause of urinary tract infection (UTI) is the Gram-negative, facultative anaerobic bacterium uropathogenic *Escherichia coli*.[1] UTI affects more than half of all women during their lifetime. The costs of UTIs are estimated to be from $2.4 billion to $3.5 billion in the

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United States.[2] Most UTIs are localized infections, but in some cases, UTI can be complicated by several risk factors that can lead to pyelonephritis, bacteremia, and urosepsis. Nosocomial UTI can lead to life threatening that is a major health concern. UTIs account for 20%–30% of all the intensive care unit (ICU)-acquired infections.[3,4]

Unfortunately, with the alarming emergence of antibiotic resistance in different bacterial populations, since it undermines the antibiotic effectiveness, it has undoubtedly become a global health threat. Of particular concern, the strains of *E. coli* are often resistant to most frequently prescribed therapeutic classes of antibiotics.[1,5] Antibiotic resistance occurs due to changes in the regulation of gene expression, mutations in genes, or horizontal gene transfer, which can affect the bacterial wall construction or produce a number of antibacterial proteins.[6]

Beta-lactam-based antibiotics exhibit the most commonly prescribed therapeutic antimicrobials, and the spread and emergence of resistance to these agents has greatly increased.[5] The mode of action of β-lactam-based antibiotics in inhibition of the growth of bacteria is acylation of active-site serine in essential penicillin-binding proteins (PBPs). The general reaction and main mechanism of antimicrobial resistance in Gram-negative bacteria is commonly assumed that PBPs were the β-lactamase precursors.[7,8] β-lactamase commonality is the ability to degrade the β-lactam ring. Two structural types of β-lactamases are serine β-lactamases and metallo-β-lactamases. The most important classes of serine-β-lactamases are extended-spectrum β-lactamases (ESBLs) that could be able to hydrolyze the expanded-spectrum cephalosporins and other oxyimino-β-lactams including aztreonam, ceftazidime, and cefotaxime.[5,8] The CTX type M variants of ESBLs are the main contributor to the multidrug-resistant profile in many Gram-negative bacteria. Clearly, the CTX-M-β-lactamase enzymes are considered ESBLs which belong to the plasmid-borne Ambler's Class A and Bush–Jacoby–Medeiros group 2be. They have been grouped into five clusters based on the amino acid sequence similarities of these enzymes (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25), named after the first described member of each encoded by $bla_{CTX\text{-}M}$ genes.[5,8-10] The main types of CTX-M enzymes are CTX-M-14 and CTX-M-15 encoded by $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ genes, respectively.[10,11] Importantly, the $bla_{CTX\text{-}M\text{-}33}$ is a point mutant derivative of $bla_{CTX\text{-}M\text{-}15}$.[9,12]

The increasing rate of *E. coli*-producing ESBL infections has been documented worldwide.[13-15] Therapy of *E. coli*-producing ESBL infections is challenging, and the number of effective antimicrobial agents is limited in many countries.[16,17] Thus, advances in the development of *E. coli*-producing ESBL infection therapies have focused on the use of antimicrobial agents in combination.[18] This study mainly sought to screen UTI clinical isolates of uropathogenic *E. coli* for the spread of *E. coli*-producing ESBL in ICUs. Moreover, we investigated the antimicrobial susceptibilities to vancomycin alone and in combination with trimethoprim on the isolates of *E. coli*-producing ESBL using the broth microdilution method interpreting the synergistic effects by the fractional inhibitory concentration (FIC) index model. We have also examined the effect of vancomycin/trimethoprim combination on expression levels of $bla_{CTX\text{-}M\text{-}33}$ gene in the isolates of *E. coli*-producing ESBL.

Materials and Methods

An observational study took place at Shahid Rajaee Hospital, Gachsaran, Iran. The current study was conducted on 255 patients admitted to ICU during January 5, 2018, and June 5, 2019. An ICU-acquired UTI was a defined group of patients with a positive urine culture (at least $>10^5$ colony-forming units (CFU)/mL of one or two types of bacteria) first identified >48 h after ICU admission. The patients with negative urine culture results before ICU admission were included in the study. Written informed consent was collected from all patients, their family members, or legal guardians. The authors were removed patients' identifying information and replaced with a code, as the samples were collected from patients' data that are only related to patients' age and gender, length of ICU stay, and previous antibiotic use. All ICU patients who died in this study were excluded. This study complied with the ethical guidelines of the Declaration of Helsinki (2008). All protocols, including sampling, sample handling, and analyzing anonymized samples, were approved by the Islamic Azad University (IR.IAU.GACHSARAN.REC.1396.11).

Clinical isolates were identified as *E. coli* by colony appearance on blood agar and eosin methylene blue, cell appearance, Gram's reaction, catalase and citrate tests, H_2S production, indole, motility, methyl red and Voges–Proskauer tests, triple sugar iron agar, and urease tests. The clinical isolates were further distinguished as uropathogenic *E. coli* by the amplification of a significant virulence factor aerotaxis receptor (*aer*) gene. The obtained sequences were analyzed against the databases by BlastN with default settings and parameters. The obtained sequence was deposited to GenBank: BankIt-NCBI-NIH (https://www.Ncbi.nlm.nih.gov/WebSub/). *E. coli* ATCC 25922 was acquired from Iranian Research Organization for Science and Technology. The UTI clinical isolates were grown in Luria-Bertani broth (HiMedia Labs, India) and stored at −80°C with 20% (v/v) glycerol.[14,19,20]

The susceptibility of the clinical isolates of *E. coli* against 12 different types of beta-lactam-based antibiotics including cefixime (CFM; 5 μg/disc), cefotaxime (CTX; 30 μg/disc), ceftriaxone (CRO; 30 μg/disc), ceftazidime (CAZ; 30 μg/disc), ciprofloxacin (CIP, 5 μg/disc), gentamicin (GEN, 10 μg/disc), nalidixic acid (NAL, 30 μg/disc), nitrofurantoin (NIT; 300 μg/disc), trimethoprim (TMP; 5 μg/disc), trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 μg/disc), and vancomycin (VA; 30 μg/disk) (HiMedia Laboratories, Mumbai, India) was measured on Mueller-Hinton agar (MHA; Merck, Research Laboratories, Darmstadt, Germany) for the disc diffusion susceptibility method, according to the Clinical and Laboratory Standards Institute (CLSI)

guidelines (M02-A12).[21] The susceptibility patterns of clinical isolates of *E. coli* were analyzed using WHONET 7.12 software program [World Health Organization (WHO) Collaborating Centre for Surveillance of Antimicrobial Resistance, Boston, Massachusetts, USA].

The clinical isolates of *E. coli* were screened for production of ESBL using ESBL disc diffusion test in accordance with CLSI standards (M100-S28).[22] Disks containing ceftazidime and cefotaxime were used, and MHA plates were incubated at 35°C ± 2°C for 16–18 h. Ceftazidime zone ≤22 mm and cefotaxime zone ≤27 mm were considered indicative of the isolates of *E. coli*-producing ESBL. The phenotypic identification of the isolates of *E. coli*-producing ESBL was carried out by both ceftazidime and cefotaxime alone and in combination with clavulanate. Discs containing ceftazidime, ceftazidime-clavulanic acid (CAZ/CA 30/10 µg/disc), cefotaxime, and cefotaxime-clavulanic acid (CTX/CA 30/10 µg/disc) were aseptically placed on an inoculated MHA plates and then incubated at 35°C ± 2°C for 16–18 h. An increase in the inhibition zone diameter of ≥5 mm for either ceftazidime or cefotaxime in combination with clavulanic acid versus either agent alone was indicative of ESBL production.

For combination treatment, 2–8 × 10^4 CFU/mL of the isolates of *E. coli*-producing ESBL and two-fold dilution of vancomycin and trimethoprim (range: 0.25–512 µg/mL) alone or in combination were added to the 96-well cell culture plates and then incubated at 35°C. Subsequently, the minimal inhibitory concentration (MIC) assay was conducted by Stat Fax 303 Reader (Awareness Technology, Inc., USA) in accordance with CLSI standards (M07-A10).[23] To calculate the antimicrobial agent interactions, the FIC index model was used.[24] Results were also represented as isobolograms constructed by plotting synergistic concentrations of vancomycin and trimethoprim.

The real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR) analysis was based on the procedure previously described.[25] The inoculum of 2–8 × 10^4 CFU/mL of the isolates of *E. coli*-producing ESBL (ESBL-74) was treated with vancomycin alone and in combination with trimethoprim 2× and 1× MIC. For each treatment, the total ribonucleic acid was isolated in accordance with the RNeasy Mini Kit (Qiagen, Hilden, Germany) and treated with the RNAse-free DNase I (Fermentas, USA). RNA quality, concentrations, and absorbance ratio were measured by denaturing formaldehyde agarose gel electrophoresis 1.2% (w/v) and NanoDrop spectrophotometer ND-1000 (NanoDrop Technologies Inc., Wilmington, DE), respectively. Total cellular RNA (0.5 µg) was copied into complementary DNA (cDNA) using random hexamer oligonucleotide primer and M-MuLV reverse transcriptase (Fermentas, USA) in accordance with the manufacturer's instructions. Two oligonucleotide primer sequences (blaCTX-M-33: AY238472 and 16S ribosomal RNA (rRNA): LC314477) were generated with the Primer3 software and analyzed by the OligoAnalyzer tool (https://eu.idtdna.com/pages/tools/oligoanalyzer). The *E. coli* $bla_{CTX-M-33}$ and *16S ribosomal RNA* (*16S rRNA*) genes were amplified from the synthesized cDNA with the following oligonucleotide primers: blaCTX-M-33, 5'-CGCTCATCAGCACGATAAAG-3' and 5'-CGTCACGCTGTTGTTAGGAA-3', 16SrRNA 5'-ATGTCGATTTAGGCGCTTGT-3' and 5'-CGTTGCATCGAATTAAACCA-3'. The real-time qRT-PCR was performed using SYBR™ Green qPCR Master Mix (Fermentas, EU) in a Bio-Rad MiniOpticon™ system (USA). The cycling conditions included an initial step at 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, and subsequently annealing at 60°C for 15 s and extension at 72°C for 15 s. Eventually, the melting reaction occurred at 65°C–95°C/5 s increments. The relative expression levels of $bla_{CTX-M-33}$ gene were determined by comparative Ct method ($2^{-\Delta Ct}$ formula). Normalization was performed using the *16S rRNA* gene. Expression difference with statistically significant and a fold change of ≥2-fold or ≤0.5 were reported significant up- or downregulation, respectively.[25]

The results are presented as the three independent experiments ± standard error. Statistical analysis was performed using analysis of variance. The comparison of two means was calculated using Tukey's *post hoc* test. Differences were considered statistically significant when $P < 0.05$. Statistical analyses were performed using the SPSS 22.0 for Windows (SPSS Inc. Chicago, IL, USA).

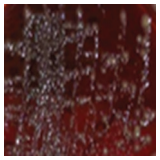
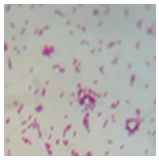
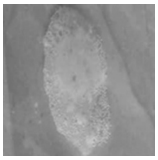
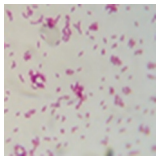
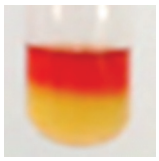
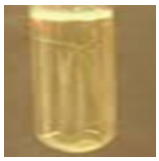
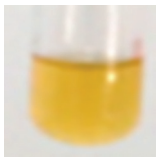
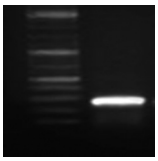
Results

Among the 255 admitted patients to the ICU, 90 clinical isolates of bacteria were isolated and identified as *E. coli* by standard laboratory methods [Table 1]. One new sequence of *E. coli* aerotaxis receptor (*aer*) gene deposited at DDBJ/EMBL/GenBank under the accession number: MN752178. The incidence rate of *E. coli* was found in 60% (n = 54/90) of clinical isolates of bacteria in female and 40% (n = 36/90) in male patients. This study included patients aged between 9 and 92 years.

The susceptibility patterns of the clinical isolates of *E. coli* are displayed in Table 2. The isolates of *E. coli* were confirmed to be resistant to vancomycin with 95% confidence interval = 94.9–100. The incidence of multidrug resistance (MDR) was 57.78% (n = 52/90) of clinical isolates of *E. coli*. Furthermore, the MDR isolates were resistant to five (n = 1/52; 1.90%), six (n = 4/52; 7.70%), seven (n = 17/52; 32.70%), and eight (n = 30/52; 57.70%) of antibiotics tested. Among clinical isolates identified as MDR *E. coli,* 100% (n = 52/52) and 48.08% (n = 25/52) were positive for possible extensively drug-resistant and possible pandrug-resistant isolates, respectively. Results showed that a total of 16.67% (n = 15/90) of the isolates of *E. coli*-producing ESBL were obtained from isolates.

The combination index assay set-up was based on CLSI broth microdilution reference method and the MIC_{90s} and FICs of vancomycin alone and in combination with trimethoprim against the isolates of *E. coli*-producing ESBL [Table 3]. Among the isolates of *E. coli*-producing ESBL, 100% (15/15) were resistant

Table 1: Morphological, biochemical, and molecular identification of *Escherichia coli* isolate

Isolate	Colony appearance (on blood agar at 37°C)	Colony appearance (on EMB agar at 37°C)	Cell appearance	Catalase test	Citrate test	Gram's reaction	H_2S production
74							
	Large, circular, gray, moist, and β-hemolytic	Green metallic sheen	Rod-shaped	Positive	Negative	Negative	Negative
	Indole test	**Motility test**	**Methyl Red test**	**TSIA**	**Urease test**	**Voges–Proskauer test**	**GenBank accession number**
							
	Positive	Positive	Positive	Acid/acid, gas positive	Negative	Negative	MN752178

EMB: Eosin methylene blue, TSIA: Triple sugar iron agar

Table 2: Antibiotic susceptibility testing results for clinical isolates of *Escherichia coli*

Code	Antibiotic name	Antibiotic class	Antibiotic subclass	Break points	Percentage resistant	Percentage intermediate	Percentage sensitive	Percentage resistant 95% CI
VAN_ND30	Vancomycin	Glycopeptide	Glycopeptide	15-16	100	0	0	94.9-100
CAZ_ND30	Ceftazidime	Cephems	Cephalosporins III	18-20	71.10	16.70	12.20	60.4-79.9
CFM_ND5	Cefixime	Cephems-oral	Cephalosporins	16-18	80	11.10	8.90	70.0-87.4
CIP_ND5	Ciprofloxacin	Quinolones	Fluoroquinolones	15-18	35.60	11.10	53.30	26.0-46.5
CRO_ND30	Ceftriaxone	Cephems	Cephalosporins III	20-22	70	14.40	15.60	59.3-79.0
CTX_ND30	Cefotaxime	Cephems	Cephalosporins III	23-25	85.60	7.80	6.70	76.3-91.8
GEN_ND10	Gentamicin	Aminoglycosides		16-19	57.80	26.70	15.60	46.9-68.0
NAL_ND30	Nalidixic acid	Quinolones	Quinolones	11-12	62.20	12.20	25.60	51.3-72.0
NIT_ND300	Nitrofurantoin	Nitrofurans		15-16	57.80	10	32.20	46.9-68.0
SXT_ND1.2	Trimethoprim/ sulfamethoxazole	Folate pathway inhibitors		11-15	42.20	10	47.80	32.0-53.1
TMP_ND5	Trimethoprim	Folate pathway inhibitors		11-15	51.10	11.10	37.80	40.4-61.7

CI: Confidence interval

to vancomycin (MIC ≥128 μg/mL) and 86.67% (n = 13/15) were resistant to trimethoprim (MIC ≥16 μg/mL). Our data showed that the vancomycin/trimethoprim combination would reduce MIC_{90} for most of the isolates of *E. coli*-producing ESBL. In comparison with vancomycin used alone, the MIC_{90s} of vancomycin were reduced 2–64-fold in concomitant use with trimethoprim. Furthermore, by comparison with trimethoprim used alone, the MICs of trimethoprim were reduced 1–2-fold in combination with vancomycin. The FIC index values of all the isolates of *E. coli*-producing ESBL were partial synergistic and indifferent effects (FIC = 0.52–1.50) in the presence of vancomycin/trimethoprim combination. Isobologram analyses showed partial synergistic and indifferent effects of two-drug combinations between vancomycin and trimethoprim against the isolates of *E. coli*-producing ESBL.

The estimated relative expression levels of $bla_{CTX-M-33}$ gene revealed significantly different ($P < 0.05$) in the isolates of *E. coli*-producing ESBL (ESBL-74) treated with vancomycin alone and in combination with trimethoprim. As seen in Figure 1, there are no significant differences in expression levels of $bla_{CTX-M-33}$ gene compared to the untreated control on vancomycin and trimethoprim challenge at both concentrations. Surprisingly, the vancomycin/trimethoprim combination treatment upon 2× and 1× MIC caused downregulation of $bla_{CTX-M-33}$ gene at negligible expression levels by 58.82- and 55.56-fold, respectively ($P \leq 0.0001$).

Discussion

E. coli is the most common cause of UTI and resulted in a considerable human death.[1,5] The rising prevalence of

Table 3: MIC (μg/mL) and FIC values and isobolograms of vancomycin and trimethoprim against isolates of *E. coli* producing ESBL

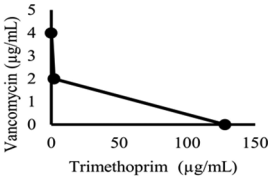
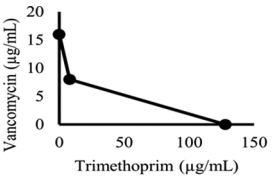
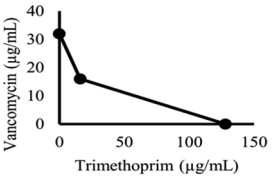
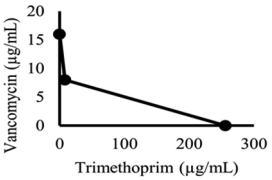
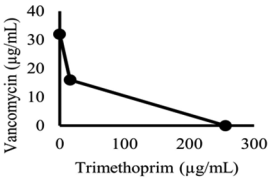
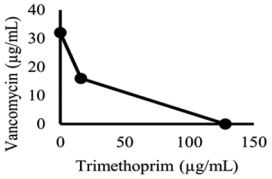
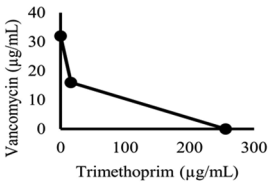
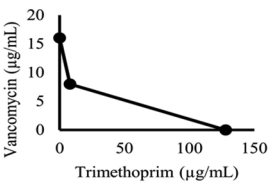
Isolates/ Antibiotics	Vancomycin	Trimethoprim	Vancomycin/Trimethoprim			Isobolograms
	MIC_{90}	MIC_{90}	MIC_{90}	FIC_{90}	Outcome	
E. coli ATCC 25922	128	4	2/2	0.52	Partial synergy	
ESBL-1	128	16	8/8	0.56	Partial synergy	
ESBL-24	128	32	16/16	0.63	Partial synergy	
ESBL-26	256	16	8/8	0.53	Partial synergy	
ESBL-32	256	32	16/16	0.56	Partial synergy	
ESBL-34	128	32	16/16	0.63	Partial synergy	
ESBL-41	256	32	16/16	0.56	Partial synergy	
ESBL-42	128	16	8/8	0.56	Partial synergy	

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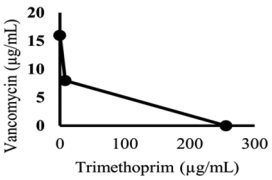
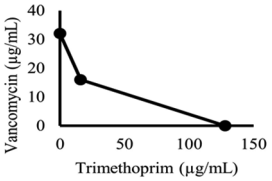
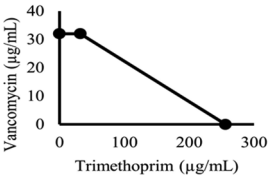
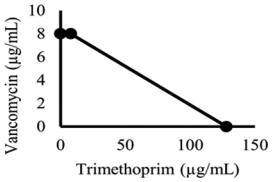
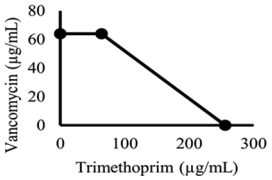
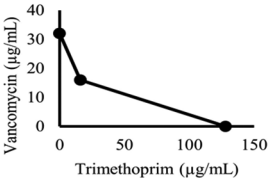
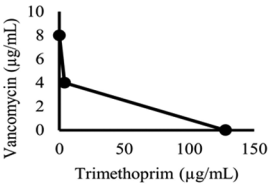
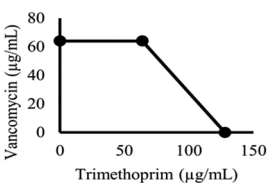
Table 3: Contd...

Isolates/ Antibiotics	Vancomycin	Trimethoprim	Vancomycin/Trimethoprim			Isobolograms
	MIC_{90}	MIC_{90}	MIC_{90}	FIC_{90}	Outcome	
ESBL-43	128	32	16/16	0.63	Partial synergy	
ESBL-44	256	16	8/8	0.53	Partial synergy	
ESBL-60	256	32	32/32	1.13	Indifferent	
ESBL-61	128	8	8/8	1.06	Indifferent	
ESBL-65	256	64	64/64	1.25	Indifferent	
ESBL-66	128	32	16/16	0.63	Partial synergy	
ESBL-74	128	8	4/4	0.53	Partial synergy	
ESBL-76	128	64	64/64	1.50	Indifferent	

ESBL: Isolates of *Escherichia coli*-producing ESBL. MIC: Minimal inhibitory concentration, ESBL: Extended-spectrum β-lactamase, FIC: Fractional inhibitory concentration, ATCC: American type culture collection

E. coli-producing ESBL has been attributed to increasing problem of antimicrobial resistance, as well as poor treatment and prevention of UTI.[5,26] In accordance with scientific papers, an alarming rise in antibiotic resistance to the isolates

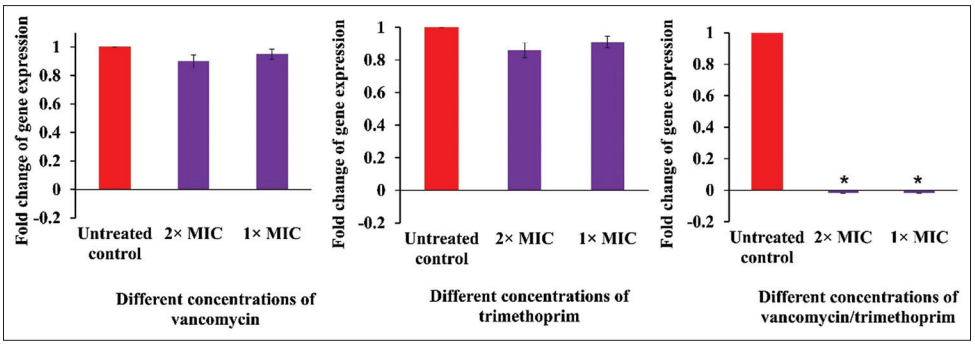


Figure 1: Gene-expression changes of $bla_{CTX-M-33}$ in the isolates of *Escherichia coli*-producing ESBL (ESBL-74) in response to vancomycin alone and in combination with trimethoprim treatment. The experiment was performed thrice, and the graph was plotted using an average value with standard error. The* denotes significant reduction ($P \leq 0.0001$) of $bla_{CTX-M-33}$ gene expression to untreated control

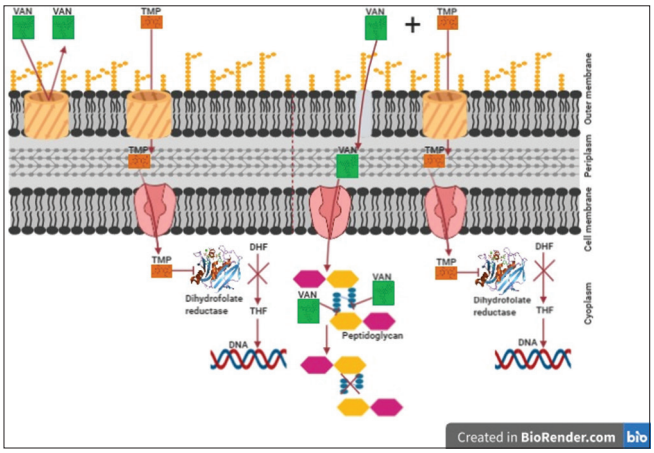


Figure 2: A model depicting how synergistic of vancomycin in combination with trimethoprim could affect the outer membrane permeability and to render vancomycin active against *Escherichia coli.* VAN: Vancomycin, TMP: Trimethoprim, DHF: Dihydrofolate, THF: Tetrahydrofolate

of *E. coli*-producing ESBL is highlighted with the results from this study. The use of antimicrobial agents in combination therapies is becoming an increasingly attractive strategy to combat antimicrobial resistance.

To date, there has been increasing interest in examining possible combination antibiotic therapy effect for treatment of *E. coli*-producing ESBL infections.[17,18] Evidence of an *in vitro* study showed promising preliminary result for the synergistic effect of vancomycin/trimethoprim combination against *E. coli.*[27]

The glycopeptide antibiotic vancomycin is a relatively large size that is greater than the exclusion limit of ß-barrel-shaped porins and thus cannot significantly penetrate the cell membrane and reach its target. Vancomycin alone is not currently used in the treatment of *E. coli* infections but only when proteins involved in outer membrane biogenesis such as SurA, Dcd, and SmpA are inhibited.[27,28] Several lines of evidence reported that combination of vancomycin with other antimicrobial molecules can expand the spectrum of useful therapeutics.[28,29] LaPlante and Sakoulas[30] examined the antibacterial activity of vancomycin alone and in combination with aztreonam or ceftazidime against *E. coli* and found that vancomycin enhanced the activates of aztreonam or ceftazidime. In the work reported here, we showed synergies between vancomycin and trimethoprim in the isolates of *E. coli*-producing ESBL. In this regard, Zhou *et al*.[27] indicated that trimethoprim sensitizes *E. coli* to vancomycin. A model depicting of synergistic mechanism of vancomycin and trimethoprim is speculated in Figure 2 (created with BioRender.com). The synergistic of vancomycin in combination with trimethoprim could enhance the entry of vancomycin into *E. coli.*

Trimethoprim is the well-known dihydrofolate reductase inhibitor, the enzyme that catalyzes the last step of folate pathway biosynthesis and thereby is an indirect inhibitor of nucleic acid synthesis.[31] The resistance rate of *E. coli* to trimethoprim has increased worldwide.[32] Resistance to trimethoprim arises through a variety of mechanisms, including modifications in dihydrofolate reductase, reduced cellular impermeability, modifications in inhibitor, and loss of binding capacity.[32,33] Evidence showed that the combined effects of trimethoprim and sulfamethoxazole on the viability of *E. coli* could enhance antibacterial against *E. coli*.[34] Several other studies have reported the effect of combinations of antimicrobial agents and trimethoprim.[35]

We have checked our results on the expression levels of $bla_{CTX-M-33}$ gene in the presence of vancomycin alone or in combination with trimethoprim in the isolates of *E. coli*-producing ESBL. Therefore, our results indicate alterations in the expression levels of $bla_{CTX-M-33}$ gene. Vancomycin/trimethoprim combination treatment showed downregulation of $bla_{CTX-M-33}$ gene. Infact, vancomycin/trimethoprim combination inhibited β-lactamases production. Consequently, extracellular-impermeable-vancomycin could be enter to the *E. coli* producing ESBL cells and may exert a bactericidal effect of trimethoprim. Our findings showed a positive correlation between synergistic effect of vancomycin/trimethoprim combination and expression of $bla_{CTX-M-33}$ gene. Kjeldsen *et al*.[36] evaluated the inhibitory growth of cefotaxime against isolates of *E. coli*-producing CTX-M-1 and investigated the expression of $bla_{CTX-M-1}$ at mRNA and protein levels. They observed that the mRNA of $bla_{CTX-M-1}$ and CTX-M-1 protein levels increased in the presence of

high cefotaxime concentrations and varied with growth phase. Brochmann *et al*.[37] investigated the effect of cefotaxime treatment on *E. coli*-producing CTX-M-15 or CMY-2 β-lactamase to analyze the differences in transcriptomes of β-lactamase genes including bla_{CMY-2}, AmpC, bla_{TEM-1}, bla_{OXA-1}, and $bla_{CTX-M-15}$. Their results showed that the transcriptional response of cefotaxime -resistant bacteria to cefotaxime were depended on the bacterial species, resistance level and resistance determinant. Singh *et al*.[38] investigated the expression of 10 β-lactamase genes of ESBL (TEM, SHV, CTX, and OXA), metallo-β-lactamases (NDM-1, IMP, and VIM), and AmpC β-lactamases (ACT, DHA, and CMY) in drug-resistant and sensitive diarrheagenic *E. coli* treated with 16 antimicrobial agents. A positive correlation was found between the expression of genes and resistant to antibiotics. All the 10 β-lactamase genes involved high levels of expression in resistant isolates of *E. coli*.

Conclusion

This *in vitro* study provides preliminary evidence of a potential synergist activity of vancomycin/trimethoprim combination against isolates of *E. coli*-producing ESBL demonstrating the downregulation of $bla_{CTX-M-33}$ gene in the isolates of *E. coli*-producing ESBL treated with vancomycin/trimethoprim combination. Further investigation needs to be conducted to ascertain whether these special events reflect the vancomycin/trimethoprim combination potential for inhibition of β-lactamase gene in ESBL-producing clinical isolates of *E. coli*. The present research demonstrated that the vancomycin/trimethoprim combination may diminish resistance in the *E. coli*-producing ESBL and could be of great importance for the development of therapeutic approaches.

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Conflicts of interest

There are no conflicts of interest.

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