

Antibiotic Resistant Genes in Multidrug-Resistant Extended-Spectrum β -lactamase-Producing *E. coli* Isolated from Children's Samples

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Abstract

Antimicrobial resistance was declared by the World Health Organization (WHO) in 2014 as the greatest threat for human and veterinary medicine. The development of resistance in *E. coli* may be due to haphazard use of antibiotics, plasmid-mediated genes, i.e. *bla*CTX-M, *bla*SHV, *bla*OXA. This work was aimed to detect the antibiotic resistant genes in multidrug-resistant extended-spectrum β -lactamase-producing *E. coli* from samples of children. 90 samples from children were collected. *bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX-M and *bla*VEB resistance genes in drug-resistant *E. coli* were investigated. The *bla*TEM, *bla*SHV and *bla*CTX genes were detected in *E. coli* isolates. *bla*TEM genes were found to be in all isolates. The *bla*SHV gene was detected in 14.28% of the isolates. The *bla*CTX-M gene was detected in 71.42% of the isolates. *bla*OXA and *bla*VEB genes were not detected in any sample. The resistant genes sequences of the *E. coli* isolates from urine, blood, skin and endotracheal tube were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases. The resistance genes sequences of the *Escherichia* isolates are greatest closely associated to those of *Escherichia coli*.

Keywords: *E. coli*; MDR; ESBLs; Children.

1. Introduction

Escherichia coli is the most common pathogens for most infections, particularly in developing countries, it caused a number of common bacterial infections including urinary tract infections, gastroenteritis and mainly neonatal meningitis. Also, *E. coli* is a major bacterial cause of Diarrhea which has been reported as leading cause of pediatric death worldwide. The Treatment of bacterial diarrhea and urinary tract infection has employed antibiotics [1,2].

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1.1 Prevalence of multi-drug resistant *E. coli*

The massive use of antibiotics, especially third generation cephalosporins, has been reported to be one of the factors contributing to the emergence and spread of bacterial resistance [3,4,5]. The spread of Infection with multidrug resistant bacteria is a global threat. Because it affects the treatment options for common infectious diseases, Nowadays, multidrug resistant bacteria are common in community-acquired infections as well and hospital-acquired infections [6]. the most common multidrug resistant organisms are Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, ESBL is a type of beta-lactamases which are able to hydrolyze wide range of cephalosporins, beta-lactams and penicillins, ESBL enzymes have spread in part due to the transmission of mobile genetic elements between bacteria. The distribution of ESBL enzymes had led to outbreaks globally [7,8]. The prevalence of the ESBL genotypes varied by times and geographical regions. In several countries the Increase of drug-resistance incidence has been reported, most of the studies showed that the rates often doubling or more over 2 to 4 years. A British study reported that 5.2% of the isolates were ESBL producers, with an increased monthly incidence from 9.5 to 13.5 cases over a 2-year period [9]. Paediatric studies in the neonatal intensive care units reported the prevalence ranging from 4.3% to 75%. Outside the neonatal age, ESBLs among hospitalised paediatric patients have recorded spread between 18.5% and 57.1%. but the carriage in community settings is lower, ranging from 0.1% to 12.4% [10]. A Study in Kathmandu reported the varying prevalence of ESBL bacteria, in Pokhara (27.7%), in Kathmandu (11 18%), in pediatric hospital in Kathmandu (14 43%) [11]. In France, ESBL implicated in urinary tract infections in the paediatric population were stable in term of prevalence (around 5%) and species distribution (90% *E. coli*) [12]. In Bolivia and Peru several studies showed a steady rise from 0.1% in 2002 to 12.4% in 2011 [13,14,15]. However, in Senegal ESBL-producing *E. coli* carriage reported among children in a very remote community, where prior antibiotic use was most unlikely, which suggest that other factors may play a role in promoting colonization [16]. This highlights the significant need for improved control practices and infection prevention and to prevent the rapid distribution of ESBL producing *E. coli*.

1.2 Genes responsible of resistance

Identification and characterization of ESBL producing *E. coli* in routine treatment of infectious diseases in pediatric patients can be particularly useful in reducing inappropriate and unnecessary antimicrobial use. ESBLs might include Class A, C or D enzymes that are mostly inhibited by clavulanic acid, the common spread mechanism is by horizontal gene transfer [5]. Class A enzymes including *TEM*, *SHV*, and *CTX-M*, and Class D enzyme *OXA* are generally found among Enterobacteriaceae [17]. The ESBLs types of *TEM* and *SHV* were detected in Europe and the United States between 1980-1990 [19,20], but *CTX-M* was spread in Asian countries [21,22]. During last decades, *CTX-M14* and *CTX-M15* were spread globally in humans and animals [23]. The recent studies reported that the cefotaxime-hydrolyzing beta-lactamase, (*CTX-M*) enzyme now predominates because of superior transmission efficiency [19]. ESBLs generality can be classified into three major groups: *TEM*, *SHV* and *CTX-M*. Worldwide, ESBL-producing bacteria are most frequently found among the members of the family Enterobacteriaceae, especially *E. coli*. Now, there are more than 60 different variants of *CTX-M*-type ESBLs, which further classified based on their amino acid sequences into five different subgroups: *CTX-M-1*, *CTX-M-2*, *CTX-M-8*, *CTX-M-9* and *CTX-M-25* groups. Of these, *CTX-M-1*, *CTX-M-2* and *CTX-M-9* groups are

the most common [24]. However, in the initial of 2000s, the dominant *CTX-M* group shifted from *CTX-M-2* to *CTX-M-9*. The β -lactamases production is an important system of resistance to β -lactam antibiotics among gram-negative bacteria. Expanded-spectrum cephalosporins have been prepared to resist degradation by the older broad-spectrum β -lactamases like *TEM-1*, *TEM-2*, and *SHV-1*. The reaction to the expanded-spectrum cephalosporins amongst the members of the Enterobacteriaceae family requiring inducible β -lactamases has been the production of mutant forms of the older β -lactamases called extended-spectrum β -lactamases (ESBL). The enzymes are able of hydrolysing the newer cephalosporins and aztreonam. Studies by biochemical and molecular techniques show that many ESBLs are derivatives of older *TEM-1*, *TEM-2*, or *SHV-1* β -lactamases, some of which differ from the parent enzyme by only one or two amino acids [25]. Several types of ESBLs have been found in many countries. The *TEM* and *SHV* types were first found in Western Europe. The *VEB* was first reported in a single isolate of *E. coli* in Vietnam [26]. Only few studies described the prevalence and the distribution of extended spectrum β -lactamase producing *E. coli*, so the detection of the resistance genes was an important need, especially among children because the wide range of serious diseases which caused by ESBL producing *E. coli*. Therefore, this study was aimed to detect the resistant genes of multidrug-resistant ESBL-producing *E. coli* isolated from children's samples.

2. Materials and Methods

2.1 Collection of samples

A total of 90 samples (from Children) were collected from patients. The samples were collected by the staffs of Microbiology Labs of hospitals. The collected samples were included 76 samples from urine, 4 samples from skin, 5 samples from endotracheal tube and 5 samples from blood.

2.2 Isolation and identification of *E. coli*

The swabs collected were streaked on MacConkey Agar plates. The plates were incubated for 24-48 hours at 37°C. The suspected *E. coli* showed red colonies. Morphological and biochemical characteristics of bacterial isolates were determined after incubation at 37°C for 24h. The bacterial isolates were characterized according to Bergey's Manual of Systematic Bacteriology [27].

2.3 Isolation of chromosomal DNA

The samples were cultured on Nutrient Agar. Thermo Scientific GeneJET Genomic DNA Purification Kit was used according to the manufacture.

2.4 Detection of resistance genes by polymerase chain reaction (PCR)

The presence of genes associated with resistance β -lactams (Amoxicillin-clavulante, Piperacillin-Tazobactam) (*blaTEM*, *blaSHV*, *blaOXA*, *blaVEB*, *blaCTX-M*) were detected by PCR. The primers (Table 1) were planned by Primer-BLAST web site [28]. GoTaq Green Master Mix kit (Promega, USA) was used. PCR reactions were achieved according to Abo-Amer and his colleagues (2018) [29]. Amplification reactions were carried out using

a DNA thermocycler (Labnet International, Model: MultigeneOpti Max). PCR products were investigated by agarose (0.7%) gel electrophoresis. The PCR products of resistance genes were sequenced (Macrogen).

Tables

Table 1: The primer sequences and predicted sizes used in the PCR

Target gene	Encoding	Oligonucleotide sequence (5'-3')	Size (bp)
<i>blaTEM</i>	β -lactamase	F: 5'-AGATCAGTTGGGTGCACGAG-3'	403
		R: 5'-TTCATTCAGCTCCGGTTCCC-3'	
<i>blaSHV</i>	β -lactamase	F:5'- CTATCGCCAGCAGGATCTGG-3'	543
		R:5'- ATTTGCTGATTTTCGCTCGGC-3'	
<i>blaOXA</i>	β -lactamase	F:5'- GCGTGTCTTTCAAGTACGGC-3'	652
		R:5'- TCTCAACCCAACCAACCCAC-3'	
<i>blaCXT-M</i>	β -lactamase	F:5'- CGCGCTACAGTACAGCGATA-3'	360
		R:5'-TCGTTGGTGGTGCCATAGTC-3'	
<i>blaVEB</i>	β -lactamase	F:5'- CCCCTCAAGACCTTTTGCCT-3'	657
		R:5'- TTCAACCCGCCATTGCCTAT -3'	

3. Results

3.1 Isolation and identification of *E. coli*

Nighty bacterial isolates were recovered from different sources of children. Bacterial isolates were recovered from children such as urine, skin, blood and endotracheal tube. According to Berge's Manual of Systematic Bacteriology, 90 bacterial isolates were described as *E. coli*. *E. coli* isolates were selected for antimicrobial susceptibility assay.

3.2 Antimicrobial resistance genes from Children isolates

Genes responsible for antimicrobial resistance were investigated in seven MDR *E. coli* isolates (resistant to 9 or more antimicrobial agents) isolates URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69, SSCH63.

Initially, the chromosomal DNA was isolated from these isolates. A volume of 5 μ l of each preparation was resolved by 0.7% agarose gel electrophoresis, confirming the presence of sufficing DNA for PCR reactions. The presence of genes (*blaTEM*, *blaSHV*, *blaOXA*, *blaCTX-M* and *blaVEB*) responsible for resistance to extended spectrum beta lactam antibiotics (ESBLs) mediate resistance to all penicillins, third generation cephalosporins

(ceftazidime, cefotaxime, and ceftriaxone) and aztreonam (except cephamycins or carbapenems); by PCR. These genes were PCR amplified of the isolates URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63. A volume of 5 µl of each PCR reaction was analyzed by 1% agarose gel electrophoresis which confirmed the PCR-products were of the expected sizes. The presence of the gene *blaTEM* was detected in all of the isolates (URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63), The gene *blaSHV* was detected in one isolate (URCH70). The gene *blaCTX-M* was detected five isolates (URCH05, URCH61, URCH58, URCH76 and SSCH63). The genes *blaOXA* and *blaVEB* were not detected in any isolate they may be present in plasmid not in chromosomal DNA (Table 2).

Table 2: Antibiotic resistance patterns for multi-resistant *Escherichia coli* isolates and the resistant genes

Isolates	Phenotype	Genotype				
		<i>blaTEM</i>	<i>blaSHV</i>	<i>blaOXA</i>	<i>blaCTX-M</i>	<i>blaVEB</i>
SSCH63	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX	+	-	-	+	-
ETTCH69	GN, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX	+	-	-	-	-
URCH05	GM, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC.	+	-	-	+	-
URCH61	EMP, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC	+	-	-	+	-
URCH58	GN, EMP, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	+	-	-	+	-
URCH76	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT	+	-	-	+	-
URCH70	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT.	+	+	-	-	-

The resistant genes sequences of the *E. coli* isolates from urine, skin, blood and endotracheal tube, were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers:

*blaCTX-M8*SSCH63 (LC431660), *blaCTX-M9*URCH05 (LC431661), *blaCTX-M10*URCH61

(LC431662), *blaCTX-M11*URCH58(LC431663), *blaCTX-M12*URCH76(LC431664), *blaTEM8*SSCH63(LC431643), *blaTEM9*ETTCH69(LC431644), *blaTEM10*URCH05(LC431645)

, *blaTEM11*URCH61(LC431646), *blaTEM12*URCH58(LC431647), *blaTEM13*URCH76(LC431648), *blaTEM14*URCH70(LC431649), *blaSHV3*URCH70(LC431652).

The nucleotide sequences of MDR bacterial isolates were compared to existing sequences in the databases. A

dendrogram demonstrating the results of resistant genes analysis is shown in Figures 1,2,3 As demonstrated.

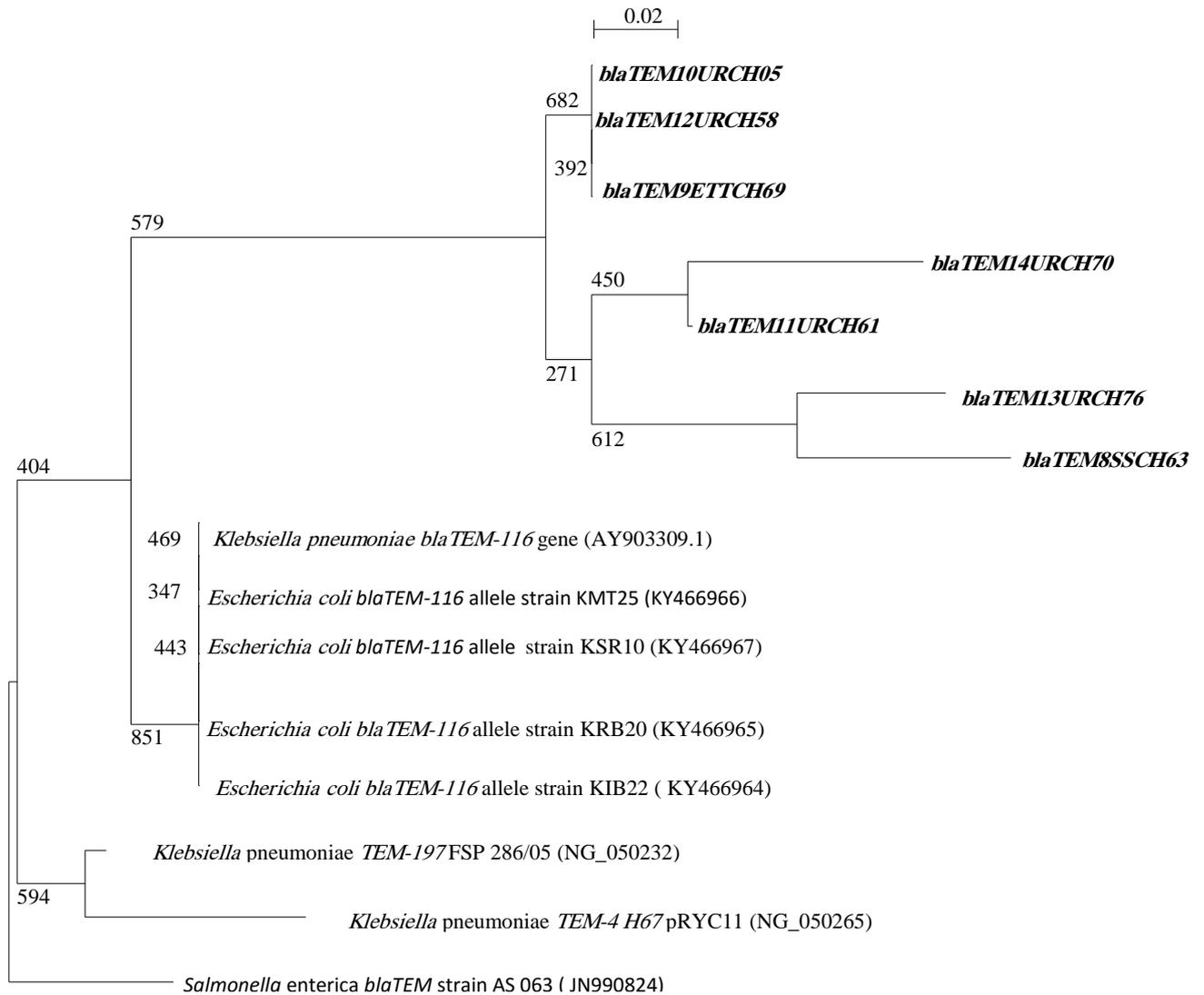


Figure 1A: phylogenetic tree of multidrug-resistant bacterial isolates based on the resistant gene (*blaTEM*) was constructed by neighbor-joining method. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates. The *Salmonella enterica* was treated as the out-group. The GenBank accession numbers of the bacteria are presented in parentheses.

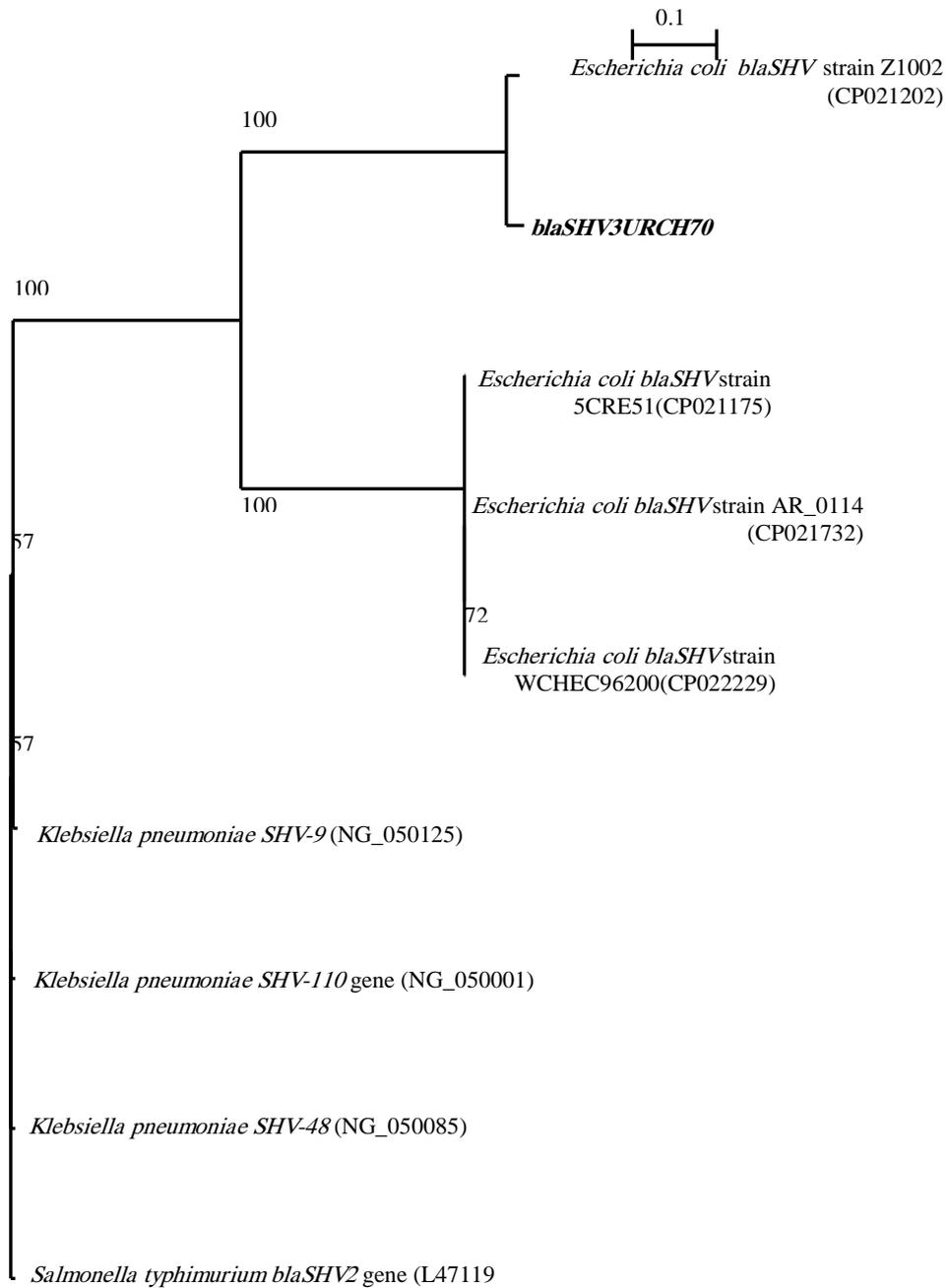


Figure 2A: phylogenetic tree of multidrug-resistant bacterial isolates based on the resistant gene (*blaSHV*) was constructed by neighbor-joining method. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates. The *Salmonella entericawas* treated as the out-group. The GenBank accession numbers of the bacteria are presented in parentheses

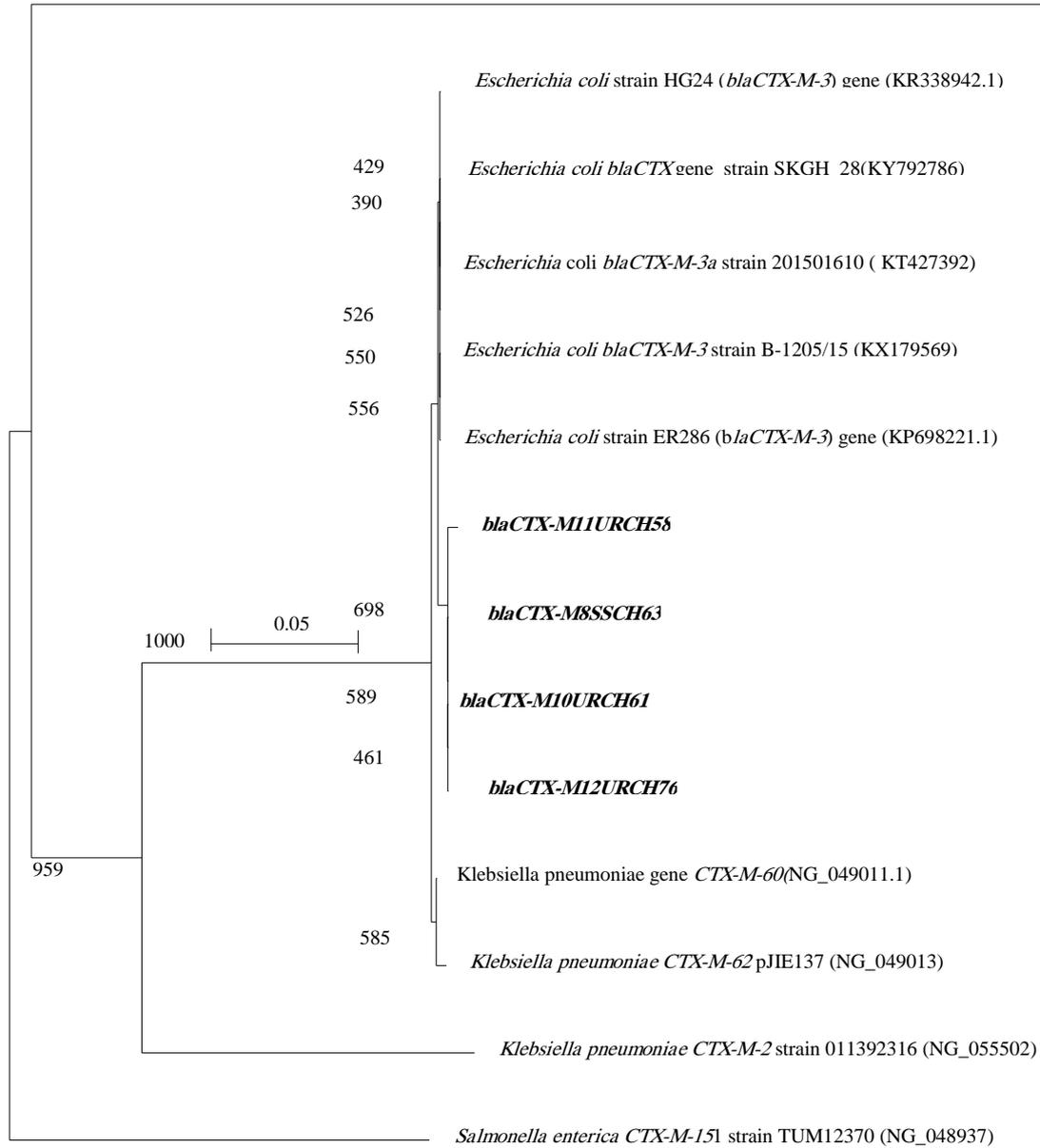


Figure 3A: phylogenetic tree of multidrug-resistant bacterial isolates based on the resistant gene (*blaCXT-M*) was constructed by neighbor-joining method. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates. The *Salmonella enterica* was treated as the out-group. The GenBank accession numbers of the bacteria are presented in parentheses

The resistant genes sequences of the *Escherichia* isolates are greatest closely associated to *Escherichia coli*. The *blaTEM* gene of isolates URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63. shares 99% similarity with that of *Escherichia coli blaTEM-116 allele* strains (KIB22, KWB6, KSR10, KMT25, KRB20). These results suggest that the isolates (URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63) are new isolates of the *Escherichia coli*. The *blaSHV* gene of isolate URCH70 shares 99% similarity with that of *Escherichia coli blaSHV* strain Z1002. These results suggest that the isolate (URCH70)

are new isolates of the *Escherichia coli*. The *blaCTX-M* gene of isolates URCH05, URCH61, URCH58, URCH76 and SSCH63. shares 99% similarity with that of *Escherichia coli blaCTX-M gene strains Escherichia coli ER286* strain. these results suggest that the isolates (URCH05, URCH61, URCH58, URCH76 and SSCH63) are new isolates of the *Escherichia coli*. These results are compatible with the conclusions of the morphological and biochemical characterization.

4. Discussion

Molecular analysis of ESBL resistant isolates indicated the presence of at least three genes that encode resistance. We observed a positive correlation between phenotypic and genotypic profiles (*SHV*, *TEM* and *CTX-M*) among our isolates, in our study, we observed diverse genotypes of ESBL among *E coli* isolates, the high spread of antimicrobial resistance and increased prevalence of ESBL-producing *E. coli* is the major findings of this study. A high prevalence of ESBL was found among pediatric patients. Identification and detection of ESBL producers in routine treatment of infectious diseases in pediatric patients can reduce unnecessary and inappropriate antimicrobial use [30]. In the present study, *blaCTX-M* gene was detected in (71.42%) among *E. coli* isolates, several studies showed that the most common resistance conferring gene was *blaCTX-M* among *E. coli* isolates obtained from children, in Canadian study from 2012 to 2017, the patients age were under 18 years, ESBL isolates showed predominance of *blaCTX-M-15* (62%) and *blaCTX-M-27* (16%) genes [31], Also, At a paediatric hospital in South Africa, found in 55.9% of *E. coli* isolates were ESBL [32]. At A Japanese children's hospital, A study Among 242 of *E. coli* isolates, 215 isolates (88.8%) were *blaCTX-M* positive [33]. In Nepal, *blaCTX-M* (66.1%) were common ESBL genotypes [34], in Iran, *blaCTX-M9* was the most common (68.2%) among isolates [35], In Qatar: all isolates harbored *blaCTX-M* gene (100%) [36]. A Nigerian study reported that 42.86% of *E. coli* isolates are *blaCTX-M* positive [37]. In this study, *blaTEM* (100%) was the most predominant genotype of ESBL among *E coli* isolates, *blaSHV* gene was detected in (14.28%), Similar to our study, in Nepal, Among MDR *E. coli* isolates showed that 40.3% from *E. coli* isolates were producing extended-spectrum β -lactamases (ESBL). *blaTEM* (83.8%), *blaSHV* (4.8%) [34], In Addition, in Tahrán A study reported that 90.9% of the *E. coli* which were Isolated from Patients with Diarrhea carried at least 1 ESBL encoding gene, *blaTEM* (54.5%) and *blaSHV* (45.4%) [35], at Nigerian paediatric hospital a study showed that the prevalence of ESBL resistance genes were: 50.00% *blaTEM* and 7.14% *blaSHV* [37]. In Qatar, Molecular analysis of resistant isolates to third generation cehalosporins indicated the presence of at least two genes that encode resistance. the study reported the positive correlation between phenotypic and genotypic profiles (*CTX-M*, *SHV* and *TEM*) in all the isolates [36]. Finally, in this study *blaOXA* and *blaVEB* were not detected in any sample, but in a study in Iran, *blaVEB* was detected in (5%) of the isolates, also in other Iranian study *blaOXA* was detected in (40.9%) of isolates [38]. In this study, the prevalence of resistant genes in ESBL- producing *E. coli* isolates in children were higher than some other investigation. The *blaTEM*, *blaSHV*, *blaCTX-M* genes which were detected in our isolates shares 99% similarity with *Escherichia coli blaTEM-116* allele strains, *Escherichia coli blaSHV* strain Z1002, *Escherichia coli ER286* strain, these results suggest that the isolates are new isolates of the *Escherichia coli*.

5. Conclusion

This proposal represents the incidence of multidrug-resistant extended-spectrum β -lactamase-producing *E. coli* among children. *E. coli* isolates positive for ESBLs phenotypically will be tested by PCR technique for ESBLs genes in this study. *blaTEM*, *blaSHV* and *blaCTX* genes were detected in *E. coli* isolates, *blaTEM* (100%), *blaSHV* (14.28%) and *blaCTX* (71.42%) in children.

6. Limitations

This study had a number of limitations. This study focused on children only, So it was difficult to obtain and collect the used isolates, since we were unable to trace the history of antibiotic prescriptions for the patients, the association between antibiotic prescription and the genotype of ESBL was unclear. also, this study was cross-sectional, we were unable to analyze the risk factors of the patients.

7. Recommendations

Our study will be a useful and helpful reference for future studies to explore and expand on the prevalence of ESBL producing *E. coli* in both children and adults in clinical and nonclinical settings, this study should cover all the hospitals in all the country to determine ESBL prevalence, in addition, Knowledge of the antimicrobial resistance patterns and resistance genes of ESBL producing *E. coli* pathogens is useful for surveillance and control of antibiotic resistance. Further research into multidrug resistance *E. coli* especially ESBL in children is essential in middle east.

8. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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