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Isolation and prevalence of multidrug-resistant extended-spectrum β-lactamase-producing *E. Coli* from adults and children

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Increasing incidence of multidrug -resistant extended-spectrum β-lactamase-producing bacteria is a significant health problem that has strongly impacted the treatment of infectious diseases and cancer. Consequently, this work was aimed to investigate the prevalence of drug-resistant *E. coli* isolated from clinical samples of adults and children.180 samples (90 from adults and 90 from children) were collected. 130 bacterial isolates (80 from children and50 from adults) showed to be *E. coli*. The prevalence of resistance to common antibiotics in adults and children was ampicillin (84%and 69%), cephalothin (72%and 54%), trimethoprim-sulfamethoxazole (62% and 47%), ciprofloxacin (54%and 24%), levofloxacin (54%and 21%), aztreonam (44%and 43%), cefepime (36%and 43%), ceftriaxone (34%and 43%), amoxicillin-clavulanate (34%and 42%), ceftazidime(32%and 43%), gentamicin (16%and 11%), nitrofurantion (4%and 3%), ertapenem(4%and 6%) and piperacillin-tazobactam (2%and 1%), respectively. MDR *E. coli* (resistance to ≥3 antimicrobial groups) in adults and children were37 (74%) and 59(73.75%), respectively. ESBL-producing rate among *E. coli* isolates was50%and 44%in adults and children, respectively. Sequences of 16S rRNA genes of the isolates share 99% similarity with that of *E. coli* strain DX15.The isolation of MDRESBL-producing *E. Coli* definitely will limit the choices of clinicians to treat their patients. Therefore, there is an urgent requisite for surveillance studies on antimicrobial resistance and incidence of ESBLs among patients to guide the clinical cure.

Keywords: *E. coli*, MDR, ESBLs, adults, children, 16SrRNA.

INTRODUCTION

Escherichia coli is primarily found in the vertebrate gut, where it is the major aerobic organism, living in symbiosis with its host (Tenaillon et al., 2010). While E. coli can transfer in water and sediment, it is frequently employed as an indicator of faecal pollution of water (Savageau et al., 1983). Pathogenic variants of *E. coli* cause much illness and death worldwide. Consequently, pathogenic *E. coli* is widely studied in humans, animals, food, and the environment. Epidemics are public in developed and developing countries, and they sometimes have serious concerns. Many of these pathotypes are a major public health concern as they have low infectious doses and are transmitted through general mediums, including food and water (Croxen et al., 2013).Furthermore, it appears that these organisms contribute significantly to delayed growth and starvation associated repeated attacks of infectious diarrhea, and similarly hungry children appear to be at higher risk of acquiring ETEC infections (Qadri et al., 2007; Petri et al., 2008).

Pathogenic strains of *E .coli* have long been recognized as agents of food borne diarrhea. It is not always appreciated that *E. coli* is an important cause of extra intestinal diseases that occur in human sites outside the gastrointestinal tract (Johnson and Russo, 2002). These include the urinary tract, central nervous system, circulatory system, and respiratory system (Russo and Johnson, 2003). There is a growing epidemic of multidrug-resistant Gram-negative pathogens and a decreasing resource of antibiotic options. Emergence of multi-resistant organisms (MROs) leads to ineffective treatment with the currently available medications which pose a great threat to public health and food technology sectors (matai et al., 2014). Antimicrobial resistance in *E. coli* has significant implications in experimental therapy, because it is associated with worse outcomes for patients with bacteraemia (Peralta et al., 2007).

Multidrug-resistant bacteria are a major component of the global AMR public health issue. Multidrug resistance (MDR) in the Gram negative family of bacteria, Enterobacteriaceae, which includes both commensal and pathogenic bacteria such as *Salmonella, E. coli,* and *Klebsiella,* is complicated by the presence of mobile genetic elements which can confer resistance and co-resistance (Exner et al., 2017).Emergence and dissemination of extended spectrum beta-lactamase (ESBL) producing *E. coli* were mostly reported from nosocomial outbreaks, but in recent days, these are also being reported from community infections as well (Oteo et al., 2010). More importantly, detection of ESBL producing *E. coli* in food producing animals and edible animal products has become a serious cause of concern for the consumers (Geser et al., 2012).Antimicrobial resistance in *E. coli* has significant implications in empirical therapy, because it is associated with worse outcomes for patients with bacteremia (Ben-Ami et al., 2009).The increase and spread of extended spectrum β-lactamase (ESBL) producing Enterobacteriaceae over the past decade has become a global problem (Ozcakar et al., 2011).In several studies, across the globe reported alarming high rate of ESBL producing *E. coli* in human infections (Cantón et al., 2008). The dynamics of antimicrobial resistance (AMR) in developing countries are poorly understood, especially in community settings, due to a sparsely of data on AMR prevalence and genetics. Therefore, this study was aimed to study the incidence of multidrug-resistant ESBL-producing *E. coli* isolated from adults and children samples at Taif province, Saudi Arabia.

MATERIALS AND METHODS

Collection of samples

A total of 130 samples (50 from adults and 80 from children) were collected from patients. The samples were collected by the staffs of Microbiology Labs of hospitals. Fifty samples were collected from adults included 36 samples from urine**,** 5 samples from wounds, 3 samples from skin**,** 2 samples from blood, 1 sample from gall bladder fluid, 2 samples from sputum and 1 sample from pus. A total of 80 samples were taken from children involved 76 samples from urine, 2 samples from skin, 1 sample from endotracheal tube and 1 sample from blood.

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^{o}C. The suspected *E. coli* showed red colonies. Morphological and biochemical characteristics of bacterial isolates were determined after incubation at 37^{o}C for 24h. The bacterial isolates were characterized according to Bergey's Manual of Systematic Bacteriology (Brenner et al., 2005).

Antimicrobial susceptibility test

Kirby Bauer Test was used (Mahon et al., 2011). A suspension of the organism following overnight growth was made to equal a 0.5 MacFarland Standard, or approximately 1.5x10 8 cfu/ml. Using a sterile swab, a lawn of the inoculum was made on Mueller Hinton agar by covering the entire surface of the plate three times. The antibiotic discs was put onto Mueller Hinton agar and incubated in a 37°C incubator for 24h. The antimicrobial agents were used as fellows: GN, gentamicin; IPM, imipenem; MEM, meropenem; FOX, cefoxitin; CTX, cefotaxime; AMP, ampicillin; AMC, amoxicillin-clavulanate; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantion; CIP, ciprofloxacin; AMK, amikacin; ETP, ertapenem; CEF, cephalothin; CXM, cefuroxime; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; CST, colistin; LVX, levofloxacin; TGC, tigecycline. The inhibition zone sizes were correlated to known MIC values of CLSI-defined breakpoints to determine whether the isolate was susceptible, intermediate, or resistant (CLSI, 2014).

Production of ESBLs by *E. coli* isolates was determined phenotypically by VITEK 2 (bioMe´rieux, Inc.). *E. coli* isolates positive for ESBLs phenotypically were tested by PCR technique for ESBLs genes.

16S rRNA characterization of *E. coli*

Isolation of chromosomal DNA

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

PCR of 16S rRNA genes

For 16S rRNA genes amplification, 27F (AGA GTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) primers were used. One microliter of template DNA was added in 20 µl of PCR reaction solution. 35 amplification cycles were achieved at 94 °C for 45 s, 55 °C for 60 s, and 72 °C for 60 s.DNA fragments were amplified ~ 1,400 bp.Un-incorporated PCR primers and dNTPs were removed from PCR products by using Montage PCR Clean up kit (Millipore).

Sequencing of 16S rRNA genes

PCR products (~1,400 bp) were sequenced by two primers: 785F (GGATTAGATACCCTGGTA) and 907R (CCGTCAATTCMTTTRAGTTT). Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA).Nominated sequences of other microorganisms with greatest match to the 16S rRNA sequences of bacterial isolates were obtained from the nucleotide sequence databases and aligned using CLUSTAL W (1.81) Multiple Sequence Alignment generating phylogenetic tree.

RESULTS

Isolation and identification of *E. coli*

One hundred and eighty bacterial isolates were recovered from different sources of adults and children. Ninety bacterial isolates were recovered from adults such as urine (no. 70), wounds (no.5), skin (no.5)**,** Blood (no.3), gall bladder fluid (no.2), sputum (no.3) and pus (no.2). Ninety bacterial isolates were obtained from children such as urine (no.76), skin (no.4), endotracheal tube (no.5) and blood (no.5). According to Berge's Manual of Systematic Bacteriology, 130 bacterial isolates were described as *E .coli* (Table 1). *E .coli* isolates was selected for antimicrobial susceptibility assay.

Antimicrobial susceptibility

The *E .coli* isolates were screened for 21 antimicrobials. The data indicated high prevalence of resistance to common antibiotics in adults such as ampicillin (84%), cephalothin (72%), trimethoprim-sulfamethoxazole (62%), ciprofloxacin (54%), levofloxacin (54%), and andaztreonam (44%). However, lowest rate of antibiotic resistance was shown in adults included cefepime and cefuroxime (36%), ceftriaxone and amoxicillin-clavulanate (34%), ceftazidime(32%), gentamicin (16%), cefoxitin (8%), nitrofurantion and ertapenem (4%), andpiperacillin-tazobactam (2%). Furthermore, in children, high incidence of antimicrobial resistance was demonstrated involved ampicillin (69%), cephalothin (54%), and trimethoprim-sulfamethoxazole (46%). Nevertheless, low incidence of antimicrobial resistance was represented in children such as aztreonam, ceftriaxone, ceftazidime, amoxicillin-clavulanate and ceftriaxone (43%), cefepime (41%), ciprofloxacin, (24%), levofloxacin (21%), gentamicin (11%), ertapenem (6%), nitrofurantion (3%), imipenem (1%), and piperacillin/tazobactam (1%).

These results indicated that ampicillin (%84) and cephalothin (%72) showed the highest rate of antibiotic resistance in adults. Moreover, ampicillin (69%) and cephalothin (54%) showed the highest rate of antibiotic resistance in children. However, ertapenem (4%) and piperacillin-tazobactam (%2) showed the lowest rate of antibiotic resistance in adults. Meanwhile, piperacillin-tazobactam and imipenem (1%) showed the lowest rate of antibiotic resistance in children. All *E. coli* isolatesfrom adult's samples were sensitive to imipenem, amikacin and meropenem while all isolates from children samples were sensitive to amikacin, meropenem and tigecycline. *E. coli* isolates (~50%) in adults were found to be extended spectrum β-lactamase (ESBL) producers. Whereas, in adults, ESBL-producing rate among *E. coli* isolates were~44%.

Most isolates in adults (74%) and children (73.75%) were resistant to 3-13 antimicrobial agents.

Table 1: Morphological and biochemical characteristics of bacteria isolated from adults and children samples.

Characteristics	*E. coli*	% of isolates	
		Adults	Children
Gram's stain	-	54	87
Rods	+	55	88
Motility	+	55	87
Catalase test	+	55	88
Oxidase test	-	54	88
Indole test	+	55	88
Methyl red	+	55	87
Voges-Proskaure	-	54	88
Citrate test	-	56	88
H_2S Production	-	55	89
Urease	-	55	87
Nitrate reduction	+	54	87
DNase production	-	56	88
Fermentation			
Glucose	+	56	88
Lactose	+	55	88
Arabinose	+	54	87
Maltose	+	56	88.5

Table 2: The prevalence of multidrug-resistant *E. coli* in adults and children.

Antimicrobials	% of antimicrobial-resistant *E. coli* isolates	
	Adults	Children
Gentamicin	16	11
Ertapenem	4	6
Cefoxitin	8	1
Ceftazidime	32	43
Ampicillin	84	69
Amoxicillin-clavulanate	34	43
Trimethoprim-sulfamethoxazole	62	46
Nitrofurantion	4	3
Ciprofloxacin	54	24
Cephalothin	72	54
Cefuroxime	36	43
Ceftriaxone	34	43
Cefepime	36	41
Aztreonam	44	43
Piperacillin-Tazobactam	2	1
Ciprofloxacin	54	24
Levofloxacin	54	21

Some MDR ESBL-producing *E. coli* isolates (resistance to 9-13 antimicrobial groups) in adults and children were selected for 16S rRNA analysis (Tables 3 and 4).

Table 3: Antimicrobial resistance patterns of some *E. coli* isolates from adults.

Isolates	Antimicrobial patterns	Number
WOAD37*****	GN, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	12
WOAD41*****	GN, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	12
WOAD40*****	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	11
URAD43*****	GN, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, CTP, LVX.	11
URAD42*****	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, CTP, LVX.	10
URAD35*****	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, CTP, LVX.	10
URAD1*****	ETP, CEF, CXM, CRO, FEP, AMP, SXT, CTP, LVX.	9

*ESBLs

Table 4: Antimicrobial resistance patterns of some *E. coli* isolates from children.

Isolates	Antimicrobial patterns	Number
URCH58*	GN, IMP, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	13
ETTCH69*	GN, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	12
SSCH63*	CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC, SXT, CTP, LVX.	11
URCH70*	CEF, CXM, CAZ, CRO, FEP, ATM, AMP, AMC, SXT.	9
URCH76*	CEF, CXM, CAZ, CRO, FEP, ATM, AMP, AMC, SXT.	9
URCH5*	GN,CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC.	9
URCH61*	IMP, CEF, CXM, CTX, CRO, FEP, ATM, AMP, AMC.	9

*ESBLs

16S rRNA analysis

Fourteen samples (7 from each adults and children) showed the highest rates of multidrug resistance were selected for 16S rRNA characterization.16S rRNA encoding genes of MDR bacterial isolates URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63 of children; and URAD35, URAD01, URAD43, URAD42, WSAD37, WSAD40 and WSAD41 of adults were PCR-amplified and sequenced. The 16S rRNA gene sequences of the bacterial isolates were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers: LC425023 (URCH05), LC425024 (URCH61), LC425025 (URCH58), LC425026 (URCH76), LC425027 (URCH70), LC425028 (SSCH63), LC425029 (ETCH69), LC425030 (URAD35), LC425031 (URAD01), LC425032 (URAD43), LC425033 (URAD42), LC425034 (WSAD37), LC425035 (WSAD40) and LC425036 (WSAD41).

16S rRNA gene analysis was applied including PCR, Sequencing and drawing generating phylogenetic tree by CLUSTAL W. The nucleotide sequences of MDR *E. coli* isolates were compared to existing sequences in the databases. A dendrogram demonstrating the results of 16S rRNA analysis was shown in Figures 1and 2. The results showed highest matching of isolates URAD35, URAD01, URAD43, URAD42, WSAD37, WSAD40, WSAD41, URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63 to members of the *Escherichia coli* group. As demonstrated, the 16S rRNA sequences of the *Escherichia coli* isolates are greatest closely associated to *E. coli*. These results are compatible with the conclusions of the morphological and biochemical characterization. The 16S rRNA genes of isolates URAD35, URAD01, URAD43, URAD42, WSAD37, WSAD40 and WSAD41 shares 99% similarity with that of *Escherichia coli* strain DX15 (KU872285), *Escherichia coli* strain 17J105 (MH919318), *Escherichia coli* strain ESC4 (LC189095), *Escherichia coli* strain DX20 (KU872290) and *Escherichia coli* strain DX18 (KU872288).These results suggest that the URAD35, URAD01, URAD43, URAD42, WSAD37, WSAD40, WSAD41, URCH05, URCH61, URCH58, URCH76, URCH70, ETCH69 and SSCH63 are new isolates of the bacterium *Escherichia coli.*

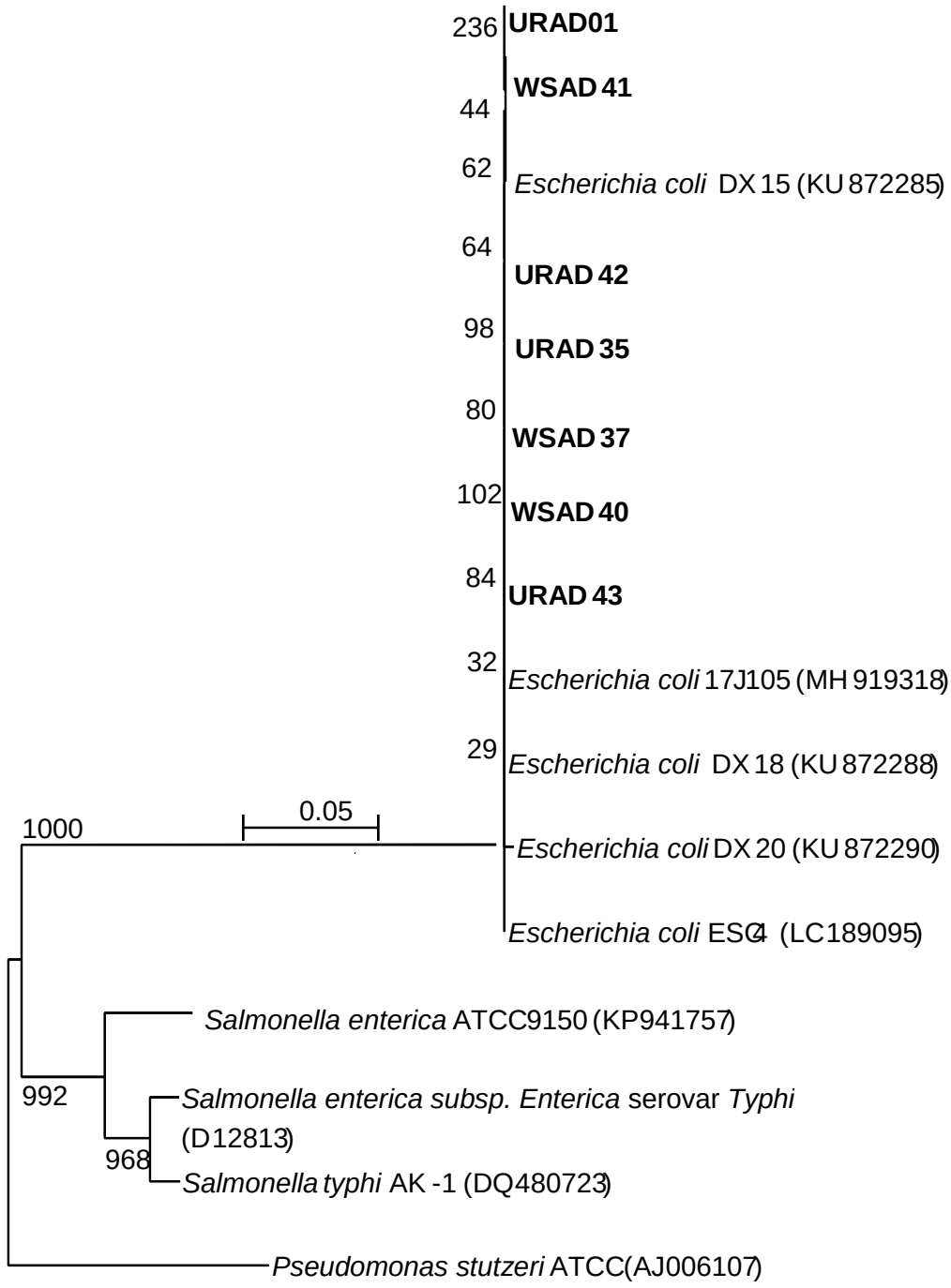

Figure 1: A phylogenetic tree of some MDRESBL-producing *E. coli* isolates from adults based on the nucleotide sequences of 16S rRNA genes was constructed by neighbour-joining method. The scale bar shows the genetic distance. The number presented next to each node shows the percentage bootstrap value of 1000 replicates. The *Pseudomonas stutzeri* was treated as the out-group. The GenBank accession numbers of the bacteria are presented in parentheses.

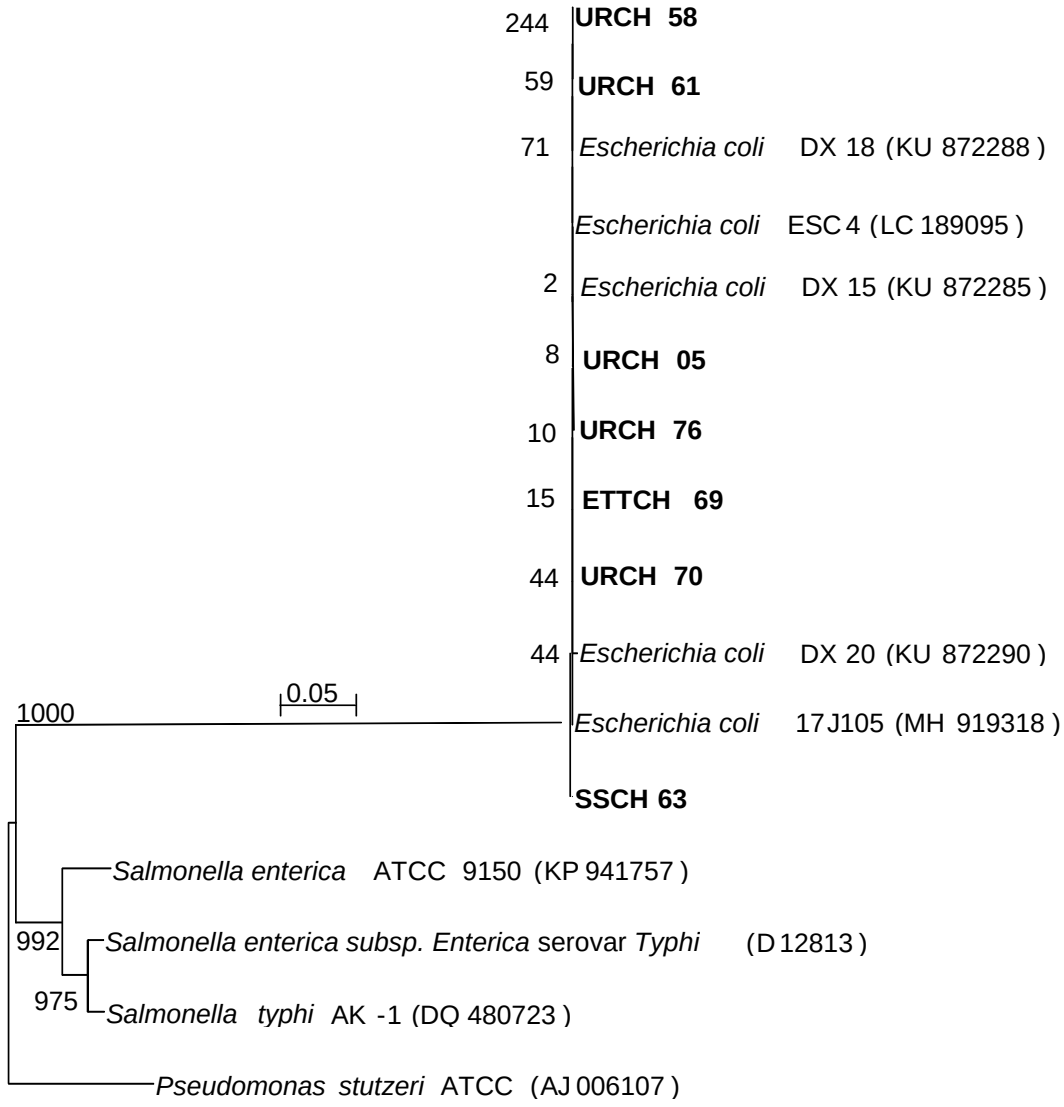

Figure 2: A phylogenetic tree of some MDR ESBL-producing *E. coli* isolates fromchildrenbased on the nucleotide sequences of 16S rRNA genes was constructed by neighbour-joining method. Other details are as for Fig. 1.

DISCUSSION

In our study 130 *E. coli* strains isolated from samples from adults and children. The highest antimicrobial resistance in adults was detected to ampicillin (84%), cephalothin (72%), trimethoprim-sulfamethoxazole (62%), Ciprofloxacin (54%), Levofloxacin (54%). Previous results of *E. coli* isolated from patients in Turkey reported that resistance to ampicillin (74%) and co-trimoxazole (61%) was significant in all isolates (Yuksel et al., 2006). Also, all *E. coli* isolates from adult's samples were sensitive to imipenem, amikacin and meropenem. Another study demonstrated that nitrofurantoin was the most active agent against *E. coli* (2% resistant isolates), followed by amikacin (5%), ceftriaxone (8%) and ciprofloxacin (12%) (Yuksel et al., 2006). In the current study, *E. coli* isolates (~50%) in adults were found to be extended spectrum β-lactamase (ESBL) producers. Also, most isolates in adults (74%) were multidrug-resistant to 3-13 antimicrobial agents. Another study in Bauchi metropolis, Nigeria, investigation of multidrug resistant (MDR) characteristics of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* pathotypes from some hospitals reported that the

antimicrobial susceptibility tests showed a high multidrug resistance among Ampicillin (97%), amoxycillin (96%), the newer generation cephalosporins like cefuroxime (81.3%), cefotaxime (85.4%) and ceftazidime (61%). The isolates were sensitive to ceftriaxone (62%), amikacin (72%) and imipenem (81%) (Iliyasu et al., 2018).

In the present study, the highest antibiotic resistance in children were noticed to ampicillin (69%), cephalothin (54%), trimethoprim-sulfamethoxazole (46%), aztreonamand Ceftriaxone (43%). In Northeast India, the prevalence of antibiotic-resistant commensal *E. coli* in pre-school and school-going children from the rural areas of Indian state stated that a high prevalence of resistance to common antibiotics like ampicillin (92%), ceftazidime (90%), cefoxitin (88%), streptomycin (40%) and tetracycline (36%), but no resistance to chloramphenicol. The resistance to the combination of penicillin and quinolone group of antibiotics was observed in fifty-two percent of the isolates (Singh et al., 2018).Moreover, in a study in north of Iran, E. col*i* isolates were obtained from paediatric patients, the ESBL-producing *E. coli* isolates were susceptible to carbapenems (66%) and amikacin (58%) and showed high resistance to cefixime (99%), colistin (82%), and ciprofloxacin (76%) (Rezai et al., 2015).Another study in In Tehran, Ilam and Mazandaran reported the highest resistance was detected to cefpodoxime (98%), trimethoprim (61%), and tetracycline (58.4%), respectively (Karami et al., 2017).Nevertheless, all *E. coli* isolates from children samples were sensitive to amikacin, meropenem and tigecycline. Another study in In Tehran, Ilam and Mazandaran reported that the highest antibiotic susceptibility of *E. coli* isolated from children was detected to imipenem (100%), followed by gentamicin (82%) and ciprofloxacin (79%) (Karami et al., 2017).

Infections with ESBL-producing bacteria were almost exclusively hospital-associated. Today, however, such infections are increasingly frequent among community-dwelling patients without a history of hospitalization or antimicrobial use (Dubois et al.,2010).In the present study, 46% *E.coli* isolates in adults were found to ESBL producers, 44% isolates which were obtained from children were found to be ESBL producers. Previous study in north of Iran reported that ESBL-producing rate among *E. coli* isolates were about 26% and 30% (Alizade*et al.,*2015 and, Rezai et al.,2015).In a recent nationwide study of ESBL-producing organisms in Spain, 51% of ESBL-producing *E. coli* strains were isolated from outpatients (Hernandez, et al.,2003).The carriage rate of ESBL-producing Enterobacteriaceae in young children in the French community setting (5%) is noteworthy, underlining the importance of this population as a reservoir (Ho et al., 2008).In Syria 26%*E. coli* isolates were found to be ESBL producers (Baaity et al., 2017), another study in Syria reviewed that 104 patients with positive urine samples to understand the risk factors for the development of ESBL infections and isolated ESBL *E. coli* in 52% of cases (Al-Assil et al., 2013).Nevertheless, the results indicated that the all isolates were sensitive to amikacin, imipenem, meropenem, cefoxitin, piperacillin-tazobactum and nitrofurantoin.

Antibiotic resistance among opportunistic pathogens is rapidly rising globally, delaying treatment of infections and increasing disease, death and health care costs (De Kraker et al., 2011). Infections with drug-resistant bacteria are associated with higher rates of illnesses and deaths, which have a serious effect on costs of health care (Tumbarello et al., 2010).The widespread use of antibiotics often without the antibiotic susceptibility testing is one of the reasons for the emergence of multidrug resistant pathogens, which seriously blocks therapeutic activities (Martinez et al., 2009). This can also delay other therapeutic successes as infectious complications appearing in patients undergoing chemotherapy for cancer or dialysis for renal failure. The effectiveness of secondary infections treatment is crucial also in surgery, especially organ transplantation (Ventola, 2015).

CONCLUSION

This proposal represents the incidence of multidrug-resistant extended-spectrum β-lactamase-producing *E. coli* among adults and children. Antimicrobials such as imipenem, amikacin and meropenem were the most effective antibiotics against ESBl-producing *E. Coli* in adults, and amikacin, meropenem and tigecycline in children. There is a crucial requirement for investigation studies on antimicrobial resistance and incidence of ESBLs among *E. coli* to guide the clinical treatment. *E. coli* isolates positive for ESBLs phenotypically will be tested by PCR technique for ESBLs genes in another study.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AEA designed the experiments and also wrote the manuscript. AEA, HM, and SS performed sample collection, isolation of bacteria, antimicrobial susceptibility, 16S rRNA analysis and data analysis. AEA and HM designed experiments and reviewed the manuscript. All authors read and approved the final version.

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