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Extended-Spectrum β-Lactamase Enzymes (ESBLs) Produced by *Escherichia coli* Urinary Pathogens at Riyadh, Saudi Arabia

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Abstract

Background: This study aim was to determine the probable type of *β-lactamase* gene which is responsible for resistance. It was found that OXA (701 bp) was the main type of *β-lactamase* (35.7%), *CTX-M* (569 bp) was second (28.9%), *TEM* (403 bp) was third (20.5%) and *SHV* (293 bp) (14.9%) was fourth.

The aim and objectives of this study were to investigate the prevalence of ESBLs producing in these bacteria isolated from uropathogenic out-patients and to look for the presence of *TEM* or *SHV*, *CTX* and *OXA* genes in *E. coli*.

Results: The present study was carried out from the Central Laboratory of Riyadh Hospital in Saudi Arabia from January 2014 to June 2015. Total 116 urine samples were tested bacteriologically and for antibiotic susceptibility using standard procedures, Detection of extended-spectrum β-lactamases and determination of the genotype of β-lactamase of 75 *E. coli* isolates by PCR: It was found that *OXA* (701 bp) was the main type of *β-lactamase* (35.7%), *CTX-M* (569 bp) was second (28.9%), *TEM* (403 bp) was third (20.5%) and *SHV* (293 bp) (14.9%) was fourth.

Conclusions: This study showed that the ESBL producing isolates detected PCR with oligonucleotide primers of *TEM*, *SHV*, and *CTX-M* and *OXA* genes and were carried out on *E. coli* DNA of 75 isolates. PCR, incorporating the primers for commonly prevalent ESBLs may be a valuable clinical and research tool for characterization of ESBLs.

Keywords: Urinary tract; Infections; Outpatients; Antibiotic susceptibility; β-lactamase; PCR

Introduction

Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed [1]. ESBLs have become widespread throughout the world and are now found in a significant percentage of *Escherichia coli* and *Klebsiella pneumonia* strains in certain countries [2]. Worldwide data show that there is increasing resistance among urinary tract pathogens to conventional drugs. *E. coli* isolates from both community and hospital infections were highly susceptible to many antimicrobial agents with the exception of those isolates producing extended spectrum β-lactamases (ESBLs) [3]. ESBL isolates are prevalent in developing countries and multiple resistant to gentamicin, ciprofloxacin, tetracycline, sulfamethoxazole/trimethoprim. They are inhibited by clavulanate (CA), sulbactam, or tazobactam [4]. More than 90% of ESBL-producing organisms were "susceptible" to cephamycins [5]. The use of cefepime to treat serious nosocomial infections (e.g., bacteremia, pneumonia, and urinary tract infections) is associated with high rates of microbiological and clinical success [6]. Treatment of extended spectrum beta-lactamase (ESBL) producing strains of Enterobacteriaceae has emerged as a major challenge in hospitalized as well as community-based patients [7].

The importance of molecular diagnostics will increase, as they are a more reliable method than phenotypic testing [8]. Plasmid mediated lactamase producing isolates of the family Enterobacteriaceae and mainly possessed the *blaTEM* (Temoneira) and the *blaCTX-M* (Cefotaximase Munchen) genes [9]. There are so many types of ESBLs like *TEM, SHV, CTX, OXA, AmpC,* etc. but the majority of the ESBLs are derivatives of TEM or SHV enzymes and these enzymes are most often found in *E. coli* and *K. pneumonia* [10]. *OXA β-lactamases* were long recognized as a less common but also a plasmid-mediated β-lactamase variety that could hydrolyze oxacillin and related anti-staphylococcal penicillins. These β-lactamases differ from the TEM and SHV (Sulphydryl variable) enzymes in that they belong to molecular class D and functional group 2d. The OXA-type β-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid [7].

The current study was investigated upon the prevalence of ESBLs producing in these bacteria isolated from uropathogenic out-patients and to look for the presence of *TEM* or *SHV*, *CTX* and *OXA* genes in *E. coli*.

Materials and Methods

Sample collection

Fresh midstream urine samples were collected from female patients 70 (60.34%) samples and 46 (39.66%) from male patients. Adult patients were sampled by clean catch midstream urine [11] and children aged less than 3 years were sampled using sterile urine bags.

Data collection

Data were conducted by a questionnaire consisting of short-answer questions including, dates, bacterial agents (first, second and third pathogen), diagnostic techniques, sex and age of patients, predisposing factors and mortality [12]. In the present study, the patients who referred to the Laboratory Center of Riyadh Hospital were studied, for a period of (January 2015 to June 2015).

Isolation and identification of organisms

The urine samples were mixed thoroughly, centrifuged and examined microscopically for wet mount preparation. This was followed by a Gram's stain. Samples for urine culture were tested within half an hour of sampling. All samples were inoculated on blood agar as well as Mac Conkey agar and incubated at 37°C for 24 h, and for 48 h in negative cases. A specimen was considered positive for UTI in the light of the number of yielded colonies (≥ 105 cfu/mL) and the cytology of the urine through microscopic detection of bacteriuria and PMNs (≥ 8 leukocytes/mm^3). However, lower colony counts associated with significant pyuria or low PMN count associated with significant colony counts was considered and analyzed in the light of the clinical picture and the patient's immunological status. Bacterial identification was based on standard culture and biochemical characteristics of isolates [13-15].

Bacterial identification

It was made using biochemical tests, namely indole, citrate, oxidase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, mannitol fermentation and novobiocin susceptibility test cystine lactose.

Electrolytes deficiency agar (CLED), analytical profile index (API) and Mueller-Hinton agar (MH).

Antimicrobial susceptibility testing by modified kirby-bauer disc diffusion method

Antibiotic susceptibility was done on Mueller-Hinton agar using disk diffusion (Kirby Bauer's) method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines using the following 21 antimicrobial agents: amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ertapenem (30 µg), nitrofurantoin (300 µg), imipenem (30 µg), meropenem (30 µg), trimethoprim/sulfamethoxazole (25 µg) [16], tigecycline (30 µg), piperacillin/tazobactam (30 µg), levofloxacin (30 µg), colistin, cephalothin, cefuroxim (10 µg), ceftriaxon (30 µg), ceftazidim (30 µg), cefoxitin (30 µg), cefepime (30 µg), aztreonam (30 µg), ampicillin (10 µg) and amoxicillin (30 µg) for all bacterial isolates (Table 1).

Aztreonam	*(ATM)*	30 µg
Ceftazidime	*(CAZ)*	30 µg
Cefepime	*(FEP)*	30 µg
Cefotaxime	*(CTX)*	30 µg
Cefpodoxime	*(POD)*	30 µg

Table 1: Antibiotics screening test for ESBLs production (Double Disc Synergy test).

Augmentin (AU) 20 µg/10 µg, with cefotaxime (CTX) 30 µg/cefpodoxime, aztreonam (ATM) 30 µg, ceftazidime (CAZ) 30 µg, and cefepime (FEP) 30 µg.

ESBL-E test

A total of 116 urine samples will be identified by using culture and sensitivity on CLED/API-strips and Mueller-Hinton agar respectively. The MICs of antibiotics were determined by the agar dilution method, as described in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines, on Mueller-Hinton agar (bioMérieux). Two agar plates will be inoculated as described for the standard disc diffusion test. An inoculum of 104 cfu/spot was applied to antibiotic-containing plates with a multipoint inoculator (West Sussex Instruments Ltd., Denley, UK). Amoxycillin was combined with the clavulanic acid in a 2:1 ratio and the concentration of tazobactam in combinations with piperacillin was 4 mg/L. The conventional double-disc test with co-amoxiclav, ceftriaxone and ceftazidime were used to detect extended-spectrum β-lactamase (ESBL) production in Enterobacteriaceae strains. Isolates with MICs of ≥ 2 mg/liter for aztreonam, ceftazidime, cefoxitin, cefotaxime and/or cefepime were checked for ESBL production by the double-disk synergy test and the E-test (AB Biodisc). For these assays, *E. coli* ATCC 25922 and *K. pneumonia* ATCC 700603 were included as quality control strains. In each plate, four 30 µg discs (aztreonam, ceftazidime, cefoxitin, cefotaxime and/or cefepime) were placed at inner disc distances (center to center) of 25 mm or 30 mm away from an amoxicillin/clavulanic acid disc (20 µg/10 µg). A clear extension of the edge of the inhibition zone towards the disc containing clavulanic acid will be interpreted as positive for ESBL production. The organisms will be tested against 3rd and 4th generation cephalosporins (aztreonam, ceftazidime, cefpodoxime, cefotaxime and\or cefepime and amoxiclav) and a second generation cephalosporins (cefoxitin) for confirmation of ESBL producer organism. The MICs which were considered to indicate susceptibility ≤ 4 µg/ml to 8 µg/ml were interpreted as susceptible, =16 µg/ml were interpreted as intermediate results and >16 were interpreted as resistant results for cefepime, cefoxitin and ceftazidime. Among, cefotaxime, aztreonam, and cefpodoxime were =2, =8 and =4 interpreted as intermediate results respectively. Also, the results interpreted as resistant were >or=4, 16 and 8 respectively.

Sampling: Sample frame: UTI patients with urosepsis.

Study duration: January 2014 to June 2015.

Validity and pre-testing

- The sterility and the efficiency of the culture media will be tested by incubating 5% of plates aerobically overnight at 37°C then check for growth.
- Control strains will be examined for growth on culture and sensitivity media.
- All reagents will be pre-tested using control strains and equipment will be calibrated Table 2.

Proteinase k	5 g
dNTPs	3000 units
Tag polymerase	3000 units
Primers (specify)	3000 units for each
$MgCl_2$ (PCR buffer)	

Electrophoresis reagents	
Agarose high grade	500 g
Ethidium bromide	5 g
Xylene cyanol	25 g
Primers for the following genes of beta-lactamases resistance	
Tem beta-Lactamases	(class A)
SHV beta-Lactamases	(class A)
CTX-M beta-Lactamases	(class A)
QXA beta-Lactamases	(class D)

Table 2: Reagents.

Quality control

The quality controls strains will be used for ESBLs testing are *K. pneumoniae* ATCC700603 as positive control and *E. coli* ATCC 25922 as a negative control. Mistakes must be checking in data entry.

Detection of extended spectrum β-lactamases: Selective testing for ESBL production was considered for all *E. coli* 75 (75%) isolates.

Plan of data analysis

The software will be used for analysis Statistical Package for Social Sciences (SPSS) program, for categorical variables proportions will be compared by the chi-square test as appropriate.

DNA extraction, PCR and sequencing

A single colony from each ESBL producing isolate was transferred into 100 µL of distilled water and the bacterial DNA was extracted by using a commercial DNA extraction kit. Bacterial genes associated with antimicrobial resistance phenotypes were detected by PCR amplification of target genes by using specific PCR primers (Table 3). The boiling method was used to extract DNA from bacterial samples [17]. *TEM, SHV, CTX-M* and *OXA β lactamase* genes were detected by a method using specific oligonucleotide primers to determine *blaTEM, blaSHV, blaCTX-M* and *blaOXA* genes. Primer sequences and their size were used for the detection of *blaTEM, blaSHV, blaCTX-M* and *blaOXA* genes in this study, which is listed in Table 3.

Primers	°C	Nucleotide seq. (5′ – 3′)	Ref (GenBank No)	Exp. Ampl size (bp)
SHV-F	60	CGCCTGTGTATTATCTCCCT	EF125011	293
SHV-R	62	CGAGTAGTCCACCAGATCCT		
TEM-F	60	TTTCGTGTCGCCCTTATTCC	AB282997	403
TEM-R	62	ATCGTTGTCAGAAGTAAGTTGG		
CTX-M-F	60	CGCTGTTGTTAGGAAGTGTG	DQ303459	569
CTX-M-R	62	GGCTGGGTGAAGTAAGTGAC		
OXA-F	64	ATGGCGATTACTGGATAGATGG	L07945	701
OXA-R	62	AGTCTTGGTCTTGGTTGTGAG		
F: Forward primer, R: Reverse primer, °C: Annealing temperature, Gene sequence of primers size bp.				

Table 3: Oligonucleotides primers used for detection of *β-lactamases* genes.

PCRs were carried out using thermal cycler (BioRad, USA) in a total volume of 25 µl containing 10 pmol of each two pair of primers (Sigma, USA), 25 µmol of dNTPs, 5 µl of template DNA, 2.5 µl of 10X Taq buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3)], 2 mM $MgCl_2$ and 2.5 U of Taq polymerase (Fermentas, USA). The Primer sequences and cycling conditions used for two different PCRs are shown in Table 3. PCR products were separated by gel electrophoresis on 1% agarose gel. In order to confirm the accuracy of genes amplified in this study, a PCR product of each gene was sent for sequencing to the Macrogen Company (South Korea) and the result was confirmed by NCBI Blast Tool.

PCR amplification of *bla* genes, including *blaTEM, blaSHV, blaCTX-M* and *bla OXA* was performed with Taq master mix DNA polymerase using primers listed in Table 3, under the following conditions.

Initial denaturation step at 95°C for 10 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C forward and 62°C reversal for 30 s for *TEM/SHV/CTX-M* genes and for *OXA* gene at 64°C forward and 62°C reversal, extension at 72°C for 2 min, followed by a final extension step at 72°C for 10 min. Respective genes were detected by the size separation-PCR amplicons by agarose gel electrophoresis.

Results

Out of 116 urine samples were collected from outpatients with urosepsis in Central Laboratory of Riyadh hospital in Saudi Arabia, during the period from January 2014 to June 2015 (Tables 1 and 2). There were 70 (60.34%) females and 46 (39.66%) males. The most commonly isolated organism was *Escherichia coli* 91(78.45%), {58 (50%) from females and 33 (28.45%) males}, Table 4.

Sex	**Female**			**Male**		
Group	Children	Young	Adult	Children	Young	Adult
Total count of 91(90 ESBL *E. coli*)	16	18	24	9	5	19
78.45%	13.79	15.52	20.69	7.76	4.31	16.38

Table 4: Total count of ESBL *E. coli* isolates on outpatients groups. ESBL *E. coli* (58 females and 23 males).

Antimicrobial susceptibility testing

Escherichia coli showed high susceptibility (98.90%) to each of amikacin, meropenem, imipenem, ertapenem and colistin. While, *E. coli* exhibited resistance to ampicillin, aztreonam, cefepime, ceftriaxone, cefuroxime, cephalothin, ceftazidime and amoxicillin.

Figure 1: *Escherichia coli*85_518F.ab1; run ended: 2015/1/23 14:37:3; signal G:6268 A:6160 C:7325 T:6271; sample: *Escherichia coli*85_518F; lane: 13; base spacing: 14.824581 1149 bases in 12439 scans.

Detection of extended-spectrum β-lactamases

The percentage of ESBL producing isolates which were reported as sensitive (S) or intermediate (I) and resistant (R) to cephalosporins were determined, Table 4. The current results showed that 90 (78.45%) of isolated *E. coli* were ESBLs producing organisms. These isolates were identified as ESBL-producers and were resistant (R) to β-lactams: ampicillin, cefazolin, ceftriaxone (MIC>64 µg/ml), aztreonam, and piperacillin. After an ESBL confirmatory test, recommended by the Clinical and Laboratory Standards Institute CLSI [18,19] showed positive results, the isolates of the present study were also considered resistant to cefotaxime, aztreonam >or=4, 16 and (MIC 16 g/mL) to cefepime.

Disk diffusion method in this study indicated of high susceptibility to cefoxitin. The ESBL producing *E. coli* strains would have been reported as sensitive for cefoxitin (87.78%), ceftazidime (46.67%), cefepime (31.11%) and for cefotaxime (5.56%). But, as intermediate for ceftazidime (21.11%), cefepime (18.89%), cefoxitin (12.22%), aztreonam (8.89%) and for cefotaxime (2.2%). Isolates were resistant for each of cefotaxime (92.22%), aztreonam (74.44%) and cefepime (50%) respectively (Table 5).

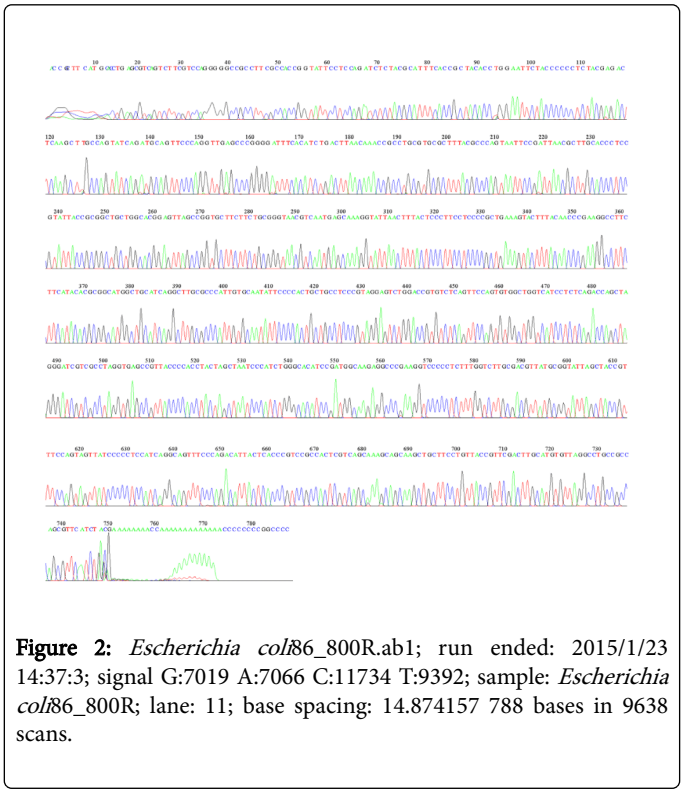

Figure 2: *Escherichia coli*86_800R.ab1; run ended: 2015/1/23 14:37:3; signal G:7019 A:7066 C:11734 T:9392; sample: *Escherichia coli*86_800R; lane: 11; base spacing: 14.874157 788 bases in 9638 scans.

Drug	**Sensitive**	**Intermediate**	**Resistant**
Cefoxitin	79 (87.78%)	11 (12.22%)	--
Aztreonam	15 (16.67%)	8 (8.89%)	67 (74.44%)
Cefotaxime	5 (5.56%)	2 (2.22%)	83 (92.22%)
Ceftazidime	42 (46.67%)	19 (21.11%)	29 (32.22%)
Cefepime	28 (31.11%)	17 (18.89%)	45 (50%)

Table 5: Susceptibility profiles of 90 ESBL-producing *E. coli* isolates.

Determination of the genotype of β-lactamase by PCR

The results of ESBL genotyping are shown in Figures 1-8 and Table 3.

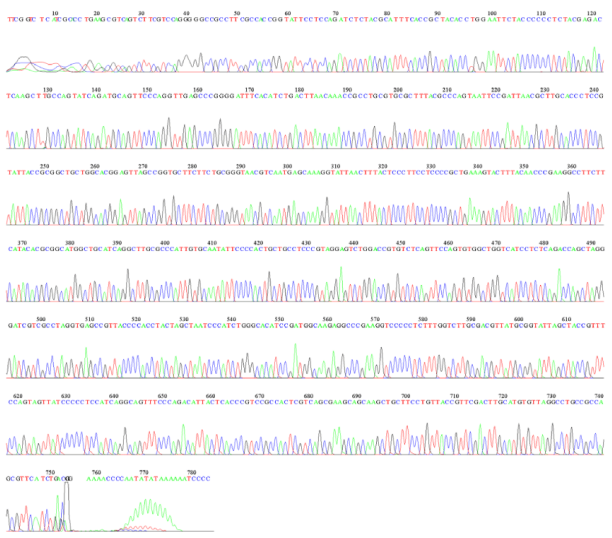

Figure 3: *Escherichia coli*95_800R.ab1; run ended: 2015/1/23 14:37:3; signal G:6199 A:6516 C:10597 T:8806; sample: *Escherichia coli*95_800R; lane: 9; base spacing: 14.864491 784 bases in 9521 scans page.

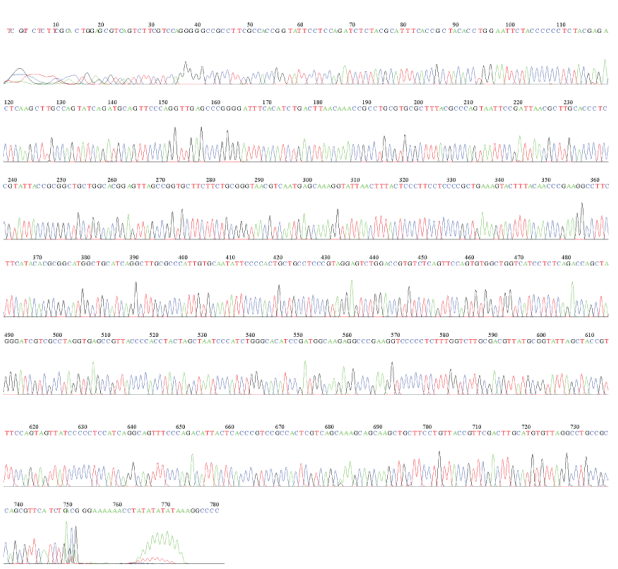

Figure 4: *Escherichia coli*97_800R.ab1; run ended: 2015/1/23 14:37:3; signal G:5542 A:6670 C:10768 T:9119; sample: *Escherichia coli*97_800R; lane: 7; base spacing: 14.928038 781 bases in 9548 scans.

Figure 5: *Escherichia coli*99_800R.ab1; run ended: 2015/1/23 14:37:3; signal G:3742 A:4552 C:7592 T:6389; sample: *Escherichia coli*99_800R; lane: 5; base spacing: 15.029872 799 bases in 9700 scans.

Figure 6: *Escherichia coli*100_518F.ab1; run ended: 2015/1/23 14:37:3; signal G:6276 A:9019 C:11357 T:9421; sample: *Escherichia coli*100_518F; lane: 3; base spacing: 15.031499 984 bases in 10922 scans.

DNA of *E. coli* isolates (75%) were analyzed by PCR. A total of 75/100 (75%) of *E. coli* isolates were confirmed to be ESBL producers. Our aim was to determine the probable type of *β-lactamase* gene which is responsible for resistance.

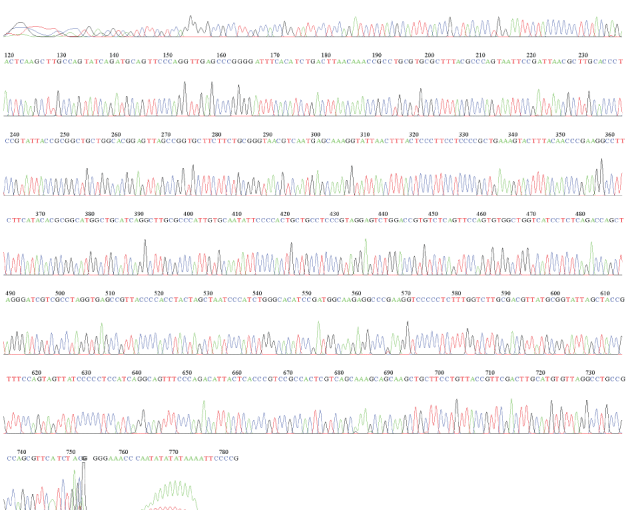

Figure 7: *Escherichia coli*100_800R.ab1; run ended: 2015/1/23 14:37:3; signal G:5287 A:6178 C:11238 T:8907; sample: *Escherichia coli*100_800R; lane: 1; base spacing: 15.2614765 783 bases in 9574 scans.

Bacterial species (50 spp.) used for specificity testing of species-specific primers Table 6. The results reveal that 38 (50.67%) *E. coli* genomes used in the design of *E. coli*-specific primers Table 7.

Identification of clinical isolates to the species level was performed on three automated identification systems; the Vitek 2 (bioMerieux, Durham, NC), the BD Pheonix (diagnostics systems, sparks, MD), and the Microscan Walkway (Siemens Healthcare Diagnostics Inc., Deerfield, IL).

It was found that *OXA* (701 bp) was the main type of *β-lactamase* (35.7%), *CTX-M* (569 bp) was second (28.9%), *TEM* (403 bp) was third (20.5%) and *SHV* (293 bp) (14.9%) was fourth Table 3.

Also, eight strains of *E. coli* with run ended 14:37:3 and lanes 7, 1, 9, 11, 15 with 781 bp, 783 bp, 784 bp, 788 bp, 791 bp but lane 3 was 984 bp and lane 13 was 1149 bp as shown in Figures 1-8.

Strain	Designation
Acinetobacter baumannii	ATCC 19606
Acinetobacter lwoffi	Clinical isolate
Achromobacter xylosoxidans	Clinical isolate
Aeromonas hydrophilia	Clinical isolate
Aeromonas veronii	Clinical isolate
Bacillus subtilis	ATCC 6633
Burkholderia cepacia	ATCC 25416
Citrobacter freundii	ATCC 8090
Citrobacter koseri	Clinical isolate
Clostridium difficile	ATCC 43255
Enterobacter aerogenes	ATCC 13048
Enterobacter cloacae	ATCC 13047
Enterobacter gergoviae	Clinical isolate
Escherichia coli	ATCC 35218
Escherichia coli O157	ATCC 43888
Enterococcus casse	Clinical isolate
Enterococcus faecalis	ATCC 51299
Enterococcus faecium	Clinical isolate
Enterococcus gallinarium	ATCC 24311
Haemophilus influenzae	ATCC 10211
Hafnia alvei	ATCC 51873
Klebsiella oxytoca	Clinical isolate
Klebsiella pneumoniae	ATCC 138
Kluyvera ascorbata	Clinical isolate
Micrococcus luteus	ATCC 53
Moraxella osloensis	ATCC 10973
Morganella morganii	Clinical isolate
Neisseria meningitidis	ATCC 53415
Pasteurella multocida	Clinical isolate
Proteus mirabilis	ATCC 12453
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas flourescens	ATCC 13525
Pseudomonas putida	Clinical isolate
Pseudomonas stutzeri	Clinical isolate
Providencia rettgeri	Clinical isolate
Providencia stuartii	MRSN 2154
Serratia marcesens	ATCC 43861
Salmonella typhi	ATCC 14028
Shigella flexneri	ATCC 12022
Staphylococcus aureus	BAA 976
Staphylococcus capitis	Clinical isolate
Staphylococcus hemolyticus	Clinical isolate
Staphylococcus epidermidis	ATCC 12228
Staphylococcus saprophyticus	ATCC 15305
Streptococcus agalactiae	ATCC 12380
Streptococcus pyogenes	ATCC 19615
Streptococcus pneumoniae	ATCC 4969
Streptococcus sanguis	ATCC 10556

Streptococcus salivaius	ATCC 13419
Stenotrophomonas maltocida	Clinical isolate

Table 6: Bacterial species (50 spp.) used for specificity testing of species-specific primers.

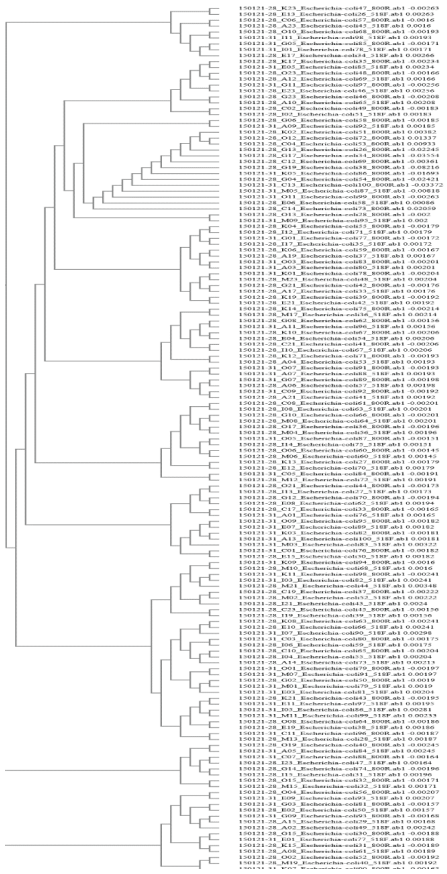

Figure 8: *E. coli* tree.

Designation	GenBank accession
Escherichia coli str.K-12 substr.MG1655	NC_000913.2
Escherichia coli O157:H7 str.EDL933	NC_002655.2
Escherichia coli O157:H7 str.Sakai	NC_002695.1
Escherichia coli UTI89	NC_007946.1
Escherichia coli 536	NC_008253.1
Escherichia coli APEC O1	NC_008563.1
Escherichia coli HS	NC_009800.1
Escherichia coli E24377A	NC_009801.1
Escherichia coli ATCC 8739	NC_010468.1
Escherichia coli str.K-12 substr.DH10B	NC_010473.1
Escherichia coli SMS-3-5	NC_010498.1
Escherichia coli O157:H7 str.EC4115	NC_011353.1
Escherichia coli SE11	NC_011415.1
Escherichia coli O127:H6 str.E2348/69	NC_011601.1
Escherichia coli IAI1	NC_011741.1
Escherichia coli S88	NC_011742.1
Escherichia coli 55989	NC_011748.1
Escherichia coli IAI39	NC_011750.1
Escherichia coli UMN026	NC_011751.1
Escherichia coli LF82	NC_011993.1
Escherichia coli BW2952	NC_012759.1
Escherichia coli B str. REL606	NC_012967.1
Escherichia coli O157:H7 str.TW14359	NC_013008.1
Escherichia coli O103:H2 str.12009	NC_013353.1
Escherichia coli O26:H11 str.11368	NC_013361.1
Escherichia coli O111:H- str.11128	NC_013364.1
Escherichia coli SE15	NC_013654.1
Escherichia coli DH1	NC_017625.1
Escherichia coli 042	NC_017626.1
Escherichia coli IHE3034	NC_017628.1
Escherichia coli ABU 83972	NC_017631.1
Escherichia coli ED1a	NC_017633.1
Escherichia coli O83:H1 str.NRG 857C	NC_017634.1
Escherichia coli NA114	NC_017644.1
Escherichia coli O7:K1 str.CE10	NC_017646.1
Escherichia coli O55:H7 str.CB9615	NC_017656.1
Escherichia coli KO11FL	NC_017660.1
Escherichia coli P12b	NC_017663.1

Table 7: List of assembled [20] *E. coli* genomes used in the design of *E. coli*-specific primers.

Discussion

Analysis of the present results according to patient sex, indicated that although, E. coli is the predominant isolated pathogen from both sexes, it occurred more frequently in females (50% in females compared to 28.45% in males). E. coli showed the highest percentage of resistance to ampicillin, aztreonam, cefepime, ceftriaxone, cefuroxime, cephalothin, ceftazidime and amoxicillin. However, all isolates of E. coli were high susceptible to meropenem, imipenem, colistin, ertapenem and amikacin. For all UTI isolates E. coli , least resistance was observed against drugs such as Ciprofloxacin and Trimethoprim/Sulfamethoxazole. This study is comparable with the

results reported by Astal and Sharif [21] and McIsaac et al. [22]. Based on the results of the present study, it was revealed that the susceptibility of bacteria to ciprofloxacin and other antibiotics were similar to many studies [21,23].

E. coli isolates producing extended spectrum β-lactamases (ESBLs) were 90 (78.45%). These isolates were identified as ESBL-producers by the double-disk synergy test and the E-test (AB Biodisc). Vercauteren et al. [24], showed that the E-test ESBL test with ceftazidime only detected 81% of ESBLs tested in their laboratory, compared to 97 and 91% for the double-disk test and the three-dimensional test, respectively. While Sanders et al. [25] showed that the Vitek ESBL test was 99% sensitive and specific for the detection of ESBLs. These data of the present study show that, by testing for ESBL results reported a significant number of ESBL producing *E. coli* strains as sensitive (S) or intermediate (I) for cefoxitin and resistant (R) or sensitive (S) and intermediate (I) for each of aztreonam, cefotaxime, ceftazidime and cefepime. The presence of an ESBL is suspected in Escherichia coli infections when resistance to one or more of the extended-spectrum cephalosporins (ESCs) (cefotaxime, ceftazidime, ceftriaxone or cefepime) is detected by [26-28]. In this study, the ESBL producing *E. coli* strains would have been reported as sensitive for cefoxitin (87.78%), ceftazidime (46.67%), cefepime (31.11%) and for cefotaxime (5.56%). But, as intermediate for ceftazidime (21.11%), cefepime (18.89%), cefoxitin (12.22%), aztreonam (8.89%) and for cefotaxime (2.2%). Isolates were resistant for each of cefotaxime (92.22%), aztreonam (74.44 %) and cefepime (50%) respectively. While, Kristo et al. [29], found that 6.4% of the ESBL producing strains were susceptible to cefotaxime, 44.6% to ceftazidime, and 55.4% to cefepime; as many as 71.8% were susceptible to at least one ESC. However, McWilliams et al. [30], recorded that *E. coli* isolates examined, 8.0%, 58.0% and 52.7% were called susceptible to cefotaxime, ceftazidime, and cefepime, respectively; All the isolates used during this study were also considered resistant to aztreonam, cefotaxime, and cefepime. But disk diffusion indicated susceptibility to cefoxitin. Cefoxitin is a cephamycin antibiotic often grouped with the second generation cephalosporins, is considered to be a strong β-lactamase inducer as are certain other antibiotics (such as imipenem), as reported by [31]. Paterson et al. [5] recorded that the cephamycins (cefoxitin, cefotetan and cefmetazole) are structurally different from the "true" cephalosporins and have enhanced stability to ESBLs. More than 90% of ESBL-producing organisms were "susceptible" to cephamycins. Tenover et al. [32] found that only 18% of laboratories correctly identified challenge organisms as potential ESBL producers using susceptibility to one or more expanded-spectrum β-lactam antibiotics as the method of detection. Changing patterns in microbial resistance suggest cefotaxime may be suffering greater resistance than ceftriaxone, whereas the two were previously considered comparable by Gums et al. [33].

PCR with oligonucleotide primers were used for detection of *TEM*, *SHV*, *CTX-M* and *OXA* genes and were carried out on DNA of 75 isolates of *E. coli*. A study by Grover et al. [34] on phenotypic and genotypic methods of ESBL detection concluded PCR. Four PCR products from different kinds of samples were sequenced during this study and reported as Saudi strains in Gen Bank (Accession Numbers: **EF125011, AB282997, DQ303459** and **L07945**. Bradford [2] showed that easiest and most common molecular method used to detect the presence of a β-lactamase belonging to a family of enzymes is PCR with oligonucleotide primers that are specific for a *β-lactamase* gene. Oligonucleotide primers can be chosen from sequences available in public databases such as Genebank. These primers are usually chosen to anneal to regions where various point mutations are not known to occur. However, PCR will not discriminate among different variants of *TEM* or *SHV*. Our molecular study revealed the ESBLs producing organisms contained *OXA* (701 bp) was the main type of *β-lactamase* (35.7%), *CTX-M* (569 bp) was second (28.9%), *TEM* (403 bp) was third (20.5%) and SHV (293 bp) (14.9%) was fourth genes by PCR. While, Thabit et al. [4] found that, CTX-M was the main type of β-lactamases, followed by *TEM*, then *SHV*. Although, the PCR data of ESBL-producing strains revealed that *blaCTX-M* genes were the most frequent *ESBL* types (74%), followed by *blaTEM* (67%) and finally *blaSHV* (45%) respectively [35]. Bradford [2] recorded that the OXA-type enzymes are another growing family of ESBLs and it was originally created as a phenotypic rather than a genotypic group for a few β-lactamases that had a specific hydrolysis profile. Therefore, there is as little as 20% sequence homology among some of the members of this family. Although, these β-lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d as reported by Thenmozhi et al. [7].

In several reports, the *TEM* gene has high frequency compared to *SHV* gene [36,37] but it was different compared to Taşli et al. [38] and Ramazanzadeh's [20] results.

In conclusion, the ESBL producing isolates detected PCR with oligonucleotide primers of *TEM, SHV*, *CTX-M* and *OXA* genes and were carried out on *E. coli* DNA of 75 isolates. PCR, incorporating the primers for commonly prevalent ESBLs may be a valuable clinical and research tool for characterization of ESBLs. Moreover, detection of *TEM, SHV, CTX-M* and *OXA* genes gave a better understanding of ESBL production [10].

Ethical Considerations

- A consent to collect the samples is obtained from different hospitals and centers included in the study.
- Valid consent of the person under the study.
- Maintaining confidentiality of information obtained from subjects under the study.
- Complete information regarding risk factors is handed to all patients under the study and no concealment what so over.
- Results of samples collected are donated to all patients included in the study and some sample results were dispatched to physicians for treatment prescription.

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