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Original paper

The β-lactamase profile of *Escherichia coli* isolates from patients with urinary tract infections in Teaching Hospital in Sulaimani, Iraq

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ABSTRACT. *Escherichia coli* bearing β-lactamase resistance genes are a leading cause of developing multi-drug resistance. The aim of this work was to study the molecular characterization and genotypic pattern of β-lactamase resistance genes in *Escherichia coli*. In total, 203 urine samples of patients who have symptoms of urinary tract infections (UTI) were screened to isolate *E. coli* and characterize resistance genes. Out of 203 patients, 32 (15.7%) cases were infected with *E. coli*. All *E. coli* samples showed a complete resistance against many antibiotics, including tetracycline (100%), rifampin (100%), and gentamycin (100%), but recorded the lowest resistance rate against imipenem (12.5%). Based on the existence of one or more gene of the chuA, yjaA and DNA fragment TSPE4.C2, *E. coli* is classified under three phylogenetic groups, A, B1, B2, and D. The highest rate of pathogenic *E. coli* was characterized under phylogenetic groups B2 (37.5%), and D (34.3%). Fifty β-lactamase resistance genes were recovered in this study and some isolates harbored more than one resistance genes. Among them, *bla*CTX recorded the highest rate, 27 (84.3%), while none of the isolates was detected to bear *bla*SHV resistance gene. Among five *bla*CMY genes, three different variants were revealed via sequencing and phylogenetic tree. Two mutations were found in one isolate at position 65 and 566, and three mutations were detected in another isolate at position number 413, 574 and 584, in comparison to a wild type variant. In conclusion, it was revealed that 15.7% cases of urinary tract infections were caused by *E. coli*. *E. coli* isolates were completely resistant to many antibiotics, but they were more sensitive to imipenem. Among the fifty β-lactamase resistance genes recovered in this study, *bla*CTX was the most common gene. There were three variants among *bla*CMY genes in a single area of study.

Keywords: *E. coli*, UTI, antibiotic resistance, β-lactamase resistance genes

Introduction

Urinary tract infection is known as one of the most common infections in hospital and in clinical practices, and *Escherichia coli* (*E. coli)* is responsible for most of the UTI [1–4]. Among people, UTI is the second common infection among people; 150 million cases are recorded annually in the world [3,5]. *E. coli* is considered a cause of hospital and community obtained UTI infections by 50% and 85%, respectively [3,6–9]. The occurrence and severity of infection is affected by many factors, such as age, gender (which is higher in female than male), genitourinary abnormalities and bacterial virulence [6,10,11]. Resistance to antibacterial medications in UTI patients is an example of increasing bacterial resistance at alarming levels and it a threat to the future of global health [12].

Emergence of multi-drug resistance in pathogenic bacteria among hospital patients is a serious health concern and it impedes the treatment, management, and controlment of the infections. β-lactam classes of antibiotics are common and effective antimicrobials that prevent and cure bacterial infections. Production of β-lactamase enzymes by bacteria is a strategy of bacteria to destroy the ring of β-lactam antibiotics and to develop resistance against antibiotics, which help bacteria to survive [13]. The increasing number of resistant bacteria against extended-spectrum

cephalosporins is related to the existence of extended-spectrum β-lactamases (ESBLs) in *E. coli,* and it alarms the serious concern of *E. coli* resistance to the new generation of β-lactam antibiotics [8,12,14–16].

There are six common and well documented β-lactamases that exist worldwide, including AmpCs, SHVs, OXAs, TEMs, and ESBLs and CTXs [17]. Extended spectrum β-lactamases originated from parent enzyme molecules but contain some mutations with a wider spectrum of activity. ESBLs are effective against first to third generations of cephalosporins, including aztreonam but not the cephamycins or carbapenems [18]. There are two types of ESBLS, classical and non-classical. Classical ESBLs are very common among *E. coli* and *Klebsiella* species, including TEM-1, TEM-2, and SHV-1 and it originated from TEM and SHV enzymes. Non-classical ESBLs are less commonly recorded than classical one in Enterobacteriacae, including OXA and CTX-M [19]. The hydrolytic activity of CTX is much greater to cefotaxim than to ceftazidime and through this way, bacteria bearing CTX gene shows a high resistance to cefotaxime [20–23].

Previous studies in the area were focused on the antibiotic sensitivity testing alone to find the pattern of antibiotic resistance in bacteria using conventional methods such as disc diffusion test, which is not very accurate [24]; therefore, depending on both sensitivity test and genetic work is more accurate. Recently, antibiotic sensitivity test has been supported by accurate methods, such as molecular biology techniques to detect antimicrobial resistance genes [24]. Therefore, in this study, we collected urine samples from patients with UTIs in Sulaimani city to isolate *E. coli*. Then, the drug resistance pattern, the existence of ESBLs, and the phylogenetic grouping of *E. coli* isolates were analyzed in order to provide the pave of using antibiotics in a reasonable way.

Materials and Methods

Sample collection and bacterial isolation

A total of 203 urine samples from patients with signs and symptoms of urinary tract infection were collected from the central diagnostic laboratory in the Teaching Hospital in Sulaimani city from November 2018 to May 2019. A loop of urine (20 ul) was inoculated on three differential medias (MacConkey agar, Blood agar, and Mannitol salt agar) (Accumedia LAB, Neogene Culture Media, Heywood, UK), and incubated at 37°C for 16 hours. For isolation of *E. coli*, a typical colony was chosen on the MacConkey agar and streaked again on eosin methylene blue (EMB) for further confirmation through development of metallic sheen green. Standard biochemical tests, including IMViC test (indole production, methyl red, Voges-Proskauer and Simon citrate test), was used for further bacterial identification and confirmation [25].

Molecular identification and confirmation of E. coli
DNA extraction

For a crude DNA extraction, a typical fresh colony was suspended in 150 ul of distilled water and the mixture was boiled at 100°C for 15 minutes. The boiled mixture was centrifuged at 10,000 RMP and two ul of the supernatant containing DNA template was used for Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction for detection of E. coli

For specific molecular detection of *E. coli*, two genes, β-glucuronidase (*uidA*) and the universal stress protein (*uspA*), were used. The flanking region of both genes *uspA* and *uidAm,* were targeted by specific primers, and the genes (Tab. 1) were amplified according to the protocol described by Heijnen [26] and Chen and Griffiths [27]. Multiplex PCR was carried out to amplify both genes in a 20 volume of CR mixture containing 10 µl of 2X premix *RedTaq* DNA polymerase (SBSbio, Beijing, China), two sets of primers (Tab. 1) (0.25 µM) and 2 µl of DNA. The PCR was run under the following conditions: 94°C for 5 min, and 35 cycles of 94°C 30 s, 55°C 30 s, 72°C 30 s, and the reaction ended with the final extension at 72°C for 7 minutes. Then the product was run on 1% agarose gel using DNA gel electrophoresis at 100 V for 30 minutes. The gel was finally visualized under blue light using SmartDoc 2.0 Imaging System (Accuris, NJ, USA)

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Phylogenetic grouping of E. coli

A single-plex PCR was performed to determine phylogenetic grouping of *E. coli* according to Bonacorsi et al. [28] with some modifications using conventional PCR. Three genes of *E. coli*, the chuA, yjaA and DNA fragment TSPE4.C2 were amplified and a combination of these genes in every strain was used to indicate the phylogenetic group of each *E. coli* isolate. Due to the small size of the genes, PCR products were resolved on 2% DNA agarose gel and

Table 1. Primer sequences of different genes used in the study

Genes	Primers	Groups of primers mixed and run in one PCR reaction
uspA	F: CCGATACGCTGCCAATCAGT	One group
	R: ACGCAGACCGTAGGCCAGAT	
uidA	F: TGGTAATTACCGACGAAAACGGC	
	R: ACGCGTGGTTACAGTCTTGCG	
blaTEM-1	F: ATAAAATTCTTGAAGACGAAA	Group 1
	R: GACAGTTACCAATGCTTAATC	
blaCMY	F: GACAGCCTCTTTCTCCACA	
	R: TGGAACGAAGGCTACGTA	
blaShv	F: TT ATCTCCCTGTTAGCCACC	Group 2
	R: GATTTGCTGATTTCGCTCGG	
blaOxa	F: TCAACTTTCAAGATCGCA	
	R: GTGTGTTTAGAATGGTGA	
blaCTX	F: CGCTTTGCGATGTGCAG	
	R: ACCGCGATATCGTTGGT	

the gel was visualized by blue light illumination using SmartDoc 2.0 Imaging System (Accuris, NJ, USA).

Determination of virulent genes in E. coli

To determine the virulent variants of *E. coli*, the following genes, *daad*, *st* (heat stable toxin), *ipaH*, *aggR*, *eaeA* (intimin), *stx1 and stx2,* were amplified and analyzed according to Guion et al. [4].

The existence of these genes in *E. coli* are an indication that the bacteria is very pathogenic and it indicates the severity of infection. Some variants with these genes cause serious complications and even death. Therefore, the amplified gene is helpful to indicate different deadly variants and pathogenic strains of *E. coli* as follow: diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), entero - invasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), entero - hemorrhagic *E. coli* (EHEC), respectively.

Polymerase Chain Reaction of β-lactamase resistance genes

Five common extended spectrum β-lactamase resistance genes (*bla*TEM-1 (1080 bp), *bla*CMY (1000 bp), *bla*Shv (800 bp), *bla*Oxa (610 bp), and *bla*CTX (550 bp)) were PCR amplified using two multiplex PCR. One multiplex was for group 1, *bla*TEM-1 *bla*CMY, and the second PCR reaction was for group 2, *bla*Shv*, bla*Oxa, and *bla*CTX (Tab. 2). In this study section, the protocol and all used primers were performed according to [29,30] with some modification. The final product was visualized under blue light after resolving on 1% DNA agarose gel using SmartDoc 2.0 Imaging System (Accuris, NJ, USA).

Sequence analysis

Seven *bla*Oxa and four *bla*CMY genes were subjected to Sanger sequencing on ABI 3730XL capillary machine (CHU de Québec-Université Laval, Québec city, Canada). All sequences have been deposited in to GenBank National Center for Biotechnology Information (NCBI) through Bankit [31] and accession numbers were obtained as listed in table 2.

The blast search of the sequences was carried out in NCBI BLASTn search tool (http://www.ncbi.nlm.nih.gov/) to compare them with similar gene sequences available online. The multi-sequence

Table 2. Accession numbers of DNA sequences of *bla*OXA and *bla*CMY recovered from different isolates of *E. coli*

Gene	Accession number	Accession number	Accession number	Accession number	Accession number	Accession number	Accession number
*bla*OXA	MN833290	MN833291	MN833292	MN833293	MN833294	MN833295	MN833296
blaCMY	MN833297	MN833298	MN833299	MN833300			

alignment was done by ClustalW multi alignment tool. A phylogenic tree was made for the local sequences and retrieved sequences of GenBank by the neighbor-joining (NJ) method (Phylogeny.fr) [32].

Antibiotic susceptibility testing

Antibacterial susceptibility test was performed according to Kirby-Bauer method (disc diffusion test) on Muller-Hinton agar (Accumedia LAB, Neogene Culture Media, and Heywood, UK). The diameter of the inhibition zone was measured around the disc, and the interpretation of the results was made according to the Clinical Laboratory Standard Institute (CLSI) guidelines [33]. The antibiotic discs used in this study were: amoxicillin (AX 25 µg), tobramycin (TOB 10 µg), amoxicillin-clavulanic acid (AMC 30 µg), tetracycline (TE 10 µg), doxycycline (DO 10 µg), imipenem (IMP 10 µg), trimethoprim/sulfamethoxazole (SXT 25 µg), meropenem (MEM 10 µg), ciprofloxacine (CIP 10 µg), gentamycin (CN 10), amikacin (AK 10 µg), nalidixic acid (NA µg), nitrofurantoin (F µg), rifampin (RA 5 µg) and cefotaxime (CTX 30 µg).

Results

Isolation and identification

In total, 203 urine samples of patients (147 females and 56 males) with signs and symptoms of UTI were collected and processed in this study. 73 (35.9%) cases were positive to bacterial culture and had urinary tract infections caused by bacteria. Of the 73 urine samples of patients with UTI, 61 (83.5%) infections were caused by a single bacterial infection, while 12 (16.4%) were caused by mixed bacterial infections. Gram-negative bacteria, 72 (98.6%) recorded the highest rate of infections among patients, while gram-positive bacteria observed only in 14 (19.1%) samples including mixed bacterial infections. Out of 203 patients, *E. coli* is the cause of 32 (15.7%) cases of UTI.

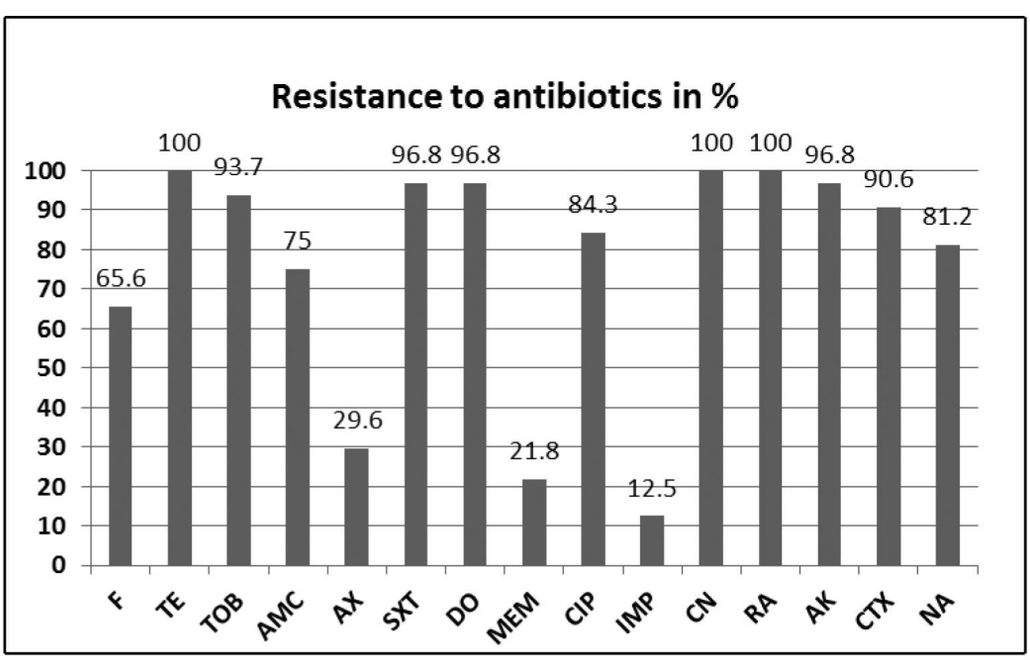

Figure 1. Antibiotic resistance pattern of *E. coli* isolates against 15 different antibiotics. The antibiotic sensitivity test was performed for *E. coli* isolates against fifteen antibiotics using Kirby Bauer test (disk diffusion test method)

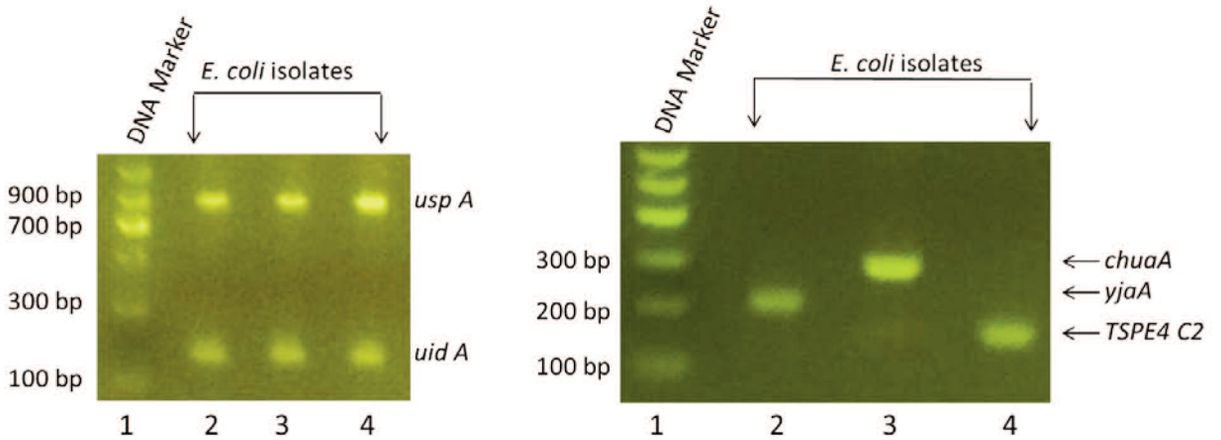

Figure 2. PCR amplification of marker genes and phylogenetic grouping of *E. coli*. *usp*A and *uid*A genes were PCR amplified and fractionated on 1% agarose gel using 1.2 Kb DNA ladder (the left image). Phylogenetic grouping was made based on chua, yjaA, and TSPE4 C2 genes (the right image). The amplicons were resolved on 2% DNA agarose gel using 0.6 Kb DNA ladder

Antibiotic susceptibility testing

All recovered *E. coli* (32 samples) from UTI were subjected to antibiotic susceptibility testing using Kirby Bauer disc diffusion test (Fig. 1). All isolates were 100% resistant to tetracycline, rifampin, and gentamycin, but the lowest resistance phenotype was to meropenem (21.8%), and against imipenem (12.5%). The resistance pattern to other antibacterial agents was as follow: doxycycline (96.8%), trimethoprim/sulfamethoxazole (96.8%), amikacin (96.8%), amoxicillin (29.6%), tobramycin (93.7%), ciprofloxacin (84.3%), cefotaxime (90.6%), nalidixic acid (81.2%), amoxicillin-clavulanic acid (75%), and nitrfurantoin (65.6%).

Molecular detection and characterization of E. coli isolates

E. coli uidA and uspA marker genes

Total, 32 isolates of *E. coli* that were recovered from UTI patients were subjected for further confirmation and molecular identification via specific gene markers of *E. coli*, *uid*A (ß-glucuronidase) and *usp*A [26,27,34]. Two specific sets of primers were used to amplify flanking regions of *uspA* and *uid*A in multiplex PCR. *E. coli* showed positive to both gene amplifications and or both of them considered as positive to *E. coli* bacteria (Fig. 2). In this study, all isolates of *E. coli* showed positive PCR amplification for both genes *USP*A and *uid*A. This molecular detection result of marker genes confirmed that all isolates were *E. coli*.

Determining phylogenetic groups of E. coli isolated from patients with UTI

In the present study, the distribution of phylogenetic groups of *E. coli* isolates in UTI cases were analyzed and characterized under four different groups, A, B1, B2, and D. The classification depended on the PCR amplification of chuA, yjaA, and part of TSPE4.C2 gene (Fig. 2). Different combination patterns of these genes indicate different phylogenetic groups as it is described by [28]. Maximum rate of *E. coli* isolates (37.5%) was characterized under group B2, and followed by group D (34.3%). The other groups, A and B1, constituted the lowest rate, (15.6%) and (12.5%), respectively.

Detection of virulent genes among E. coli isolates

E. coli strains harboring virulent genes may devastate the condition of the patients, and it may cause death in some cases due to renal failure that is caused by the development of hemolytic uremic syndrome [35]. Shiga toxin (Stx1 and stx2) genes are gene markers of STEC strains, which are responsible for serious complications such as haemolytic uremic syndrome [36]. The gene marker of the *E. coli* pathogen serovar O157:H7, enterohemorrhagic *E. coli*, is the intimin (eaeA) gene which causes severe GIT and UTI infection [37]. Therefore, it was important to determine the occurrence of the virulent genes (daad, st, ipaH, aggR, eaeA, stx1 and stx2) in different isolates of *E. coli.* This helps to choose the better treatment and control strategy. These pathogenic types of *E. coli*

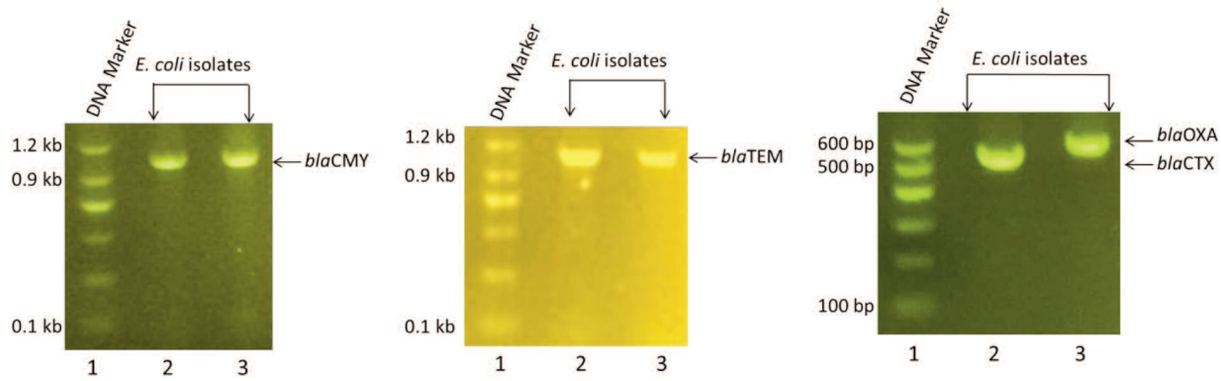

Figure 3. β-lactamase resistance genes were recovered in *E. coli*. *bla*CTX and *bla*TEM were PCR amplified using specific primers and resolved on 1% DNA agarose gel using 1.2 Kb DNA ladder (the left and middle images). *bla*CTX and *bla*OXA were PCR amplified using simplex PCR and fractionated on 2% DNA agarose gel using 0.6 Kb DNA ladder (the right image)

are known as a gastrointestinal pathogen and they usually cause diarrhea [38]. Therefore, exiting of these strains in the urinary tract may become an indication of faecal contamination.

Rapid and multiplex PCR was used to detect virulent genes in different variants of *E. coli* according to [4,34] with some modifications. In the current study, all isolates were checked by conventional PCR; none of the genes were detected in *E. coli* isolates. Therefore none of the *E. coli* isolates were detected to carry virulent genes to make a big threat to the patient's life.

Detection and molecular characterization of β-lactamase and extended spectrum β-lactamase resistance genes (ESBLs)

Recently, *E. coli* has shown a different degree of resistance to new generations of β-lactam antibiotics. β-lactamases are mostly responsible for this phenomenon, especially against cephalosporins and ampicillins. The common β-lactamase resistance genes in gram-negative bacteria, *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*OXA, and *bla*CMY-type, are well documented [17]. In the current study, an investigation was made to find the prevalence and molecular characterization of β-lactamase resistance genes in *E. coli* isolated from urine of patients who have UTI (Fig. 3). PCR amplification of coding regions of the genes using specific primers was made. *bla*CTX was the most common resistance gene observed in this study, 27 (84.3%), while *bla*SHV was not detected in any of the isolates. The second most common detected resistance genes were *bla*TEM, 9 (28.1%), *bla*OXA in 9 (28.1%), and followed by *bla*CMY2, which was observed in 5 (15.6%) isolates. 15 (46.8%) isolates were found harboring multi-resistance genes, 7 (21.8%) of them carried 3 different β-lactamase resistance genes and 8 (25%) of them had two genes. Bacteria harboring three different β-lactamase genes were found containing a combination of *bla*CTX, *bla*OXA, *bla*TEM and *bla*CTX, *bla*OXA, *bla*CMY2. *E. coli* bacteria bearing two different β-lactamase genes were revealed containing three different combinations of *bla*CTX, *bla*OXA; *bla*CTX, *bla*TEM; and *bla*CTX,

Table 3. Distribution of β-lactamase resistance genes (50 genes) on *E. coli* phylogenetic groups

Phylogenetic groups	*bla*TEM	*bla*CMY	*bla*OXA	*bla*CTX	*bla*SHV	Total
A	0	2	3	4	0	9
B1	0	0	0	2	0	2
B2	4	0	4	10	0	18
D	5	3	2	11	0	21

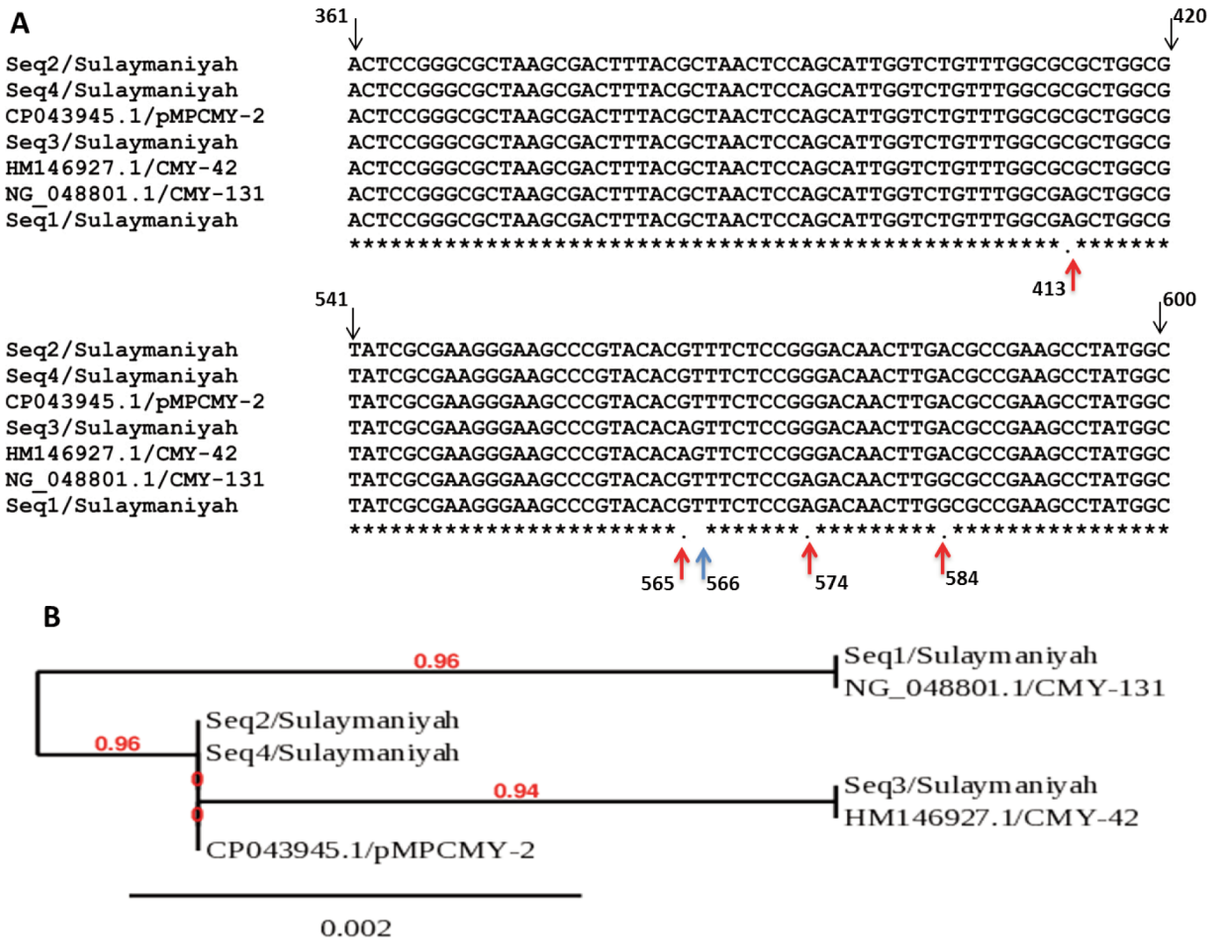

Figure 4. Phylogentic tree and sequence alignment of *bla*CMY genes recovered in *E. coli* isolates from Sulaimaniyah. A. Alignment of the gene sequences of this study with international sequences obtained from GenBank; B. Phylogenetic tree of *bla*CMY genes

*bla*CMY2. Out of 50 different β-lactamase resistance genes, 21 (42%) resistance genes were found in *E. coli* phylogenetic group D, 18 (36%) were in group B2, 9 in group A (18%), and only two isolates (4%) were found in group B1 (Tab. 3).

*bla*OXA (7 genes) and *bla*CMY (4 genes) genes where further analyzed through sequencing (Fig. 4). The local sequences were aligned to each other and then aligned to international sequences obtained from GenBank. All *bla*OXA genes were confirmed through their sequences and all of the aligned sequences of this study were identical. Whereas three different variants of *bla*CMY were reveled among 4 recovered genes (Fig. 4A). Two CTX genes completely resembled *bla*CMY2 (CP043945.1) and one CTX gene contained a single mutation indicated by blue arrow in figure 4A and it resembles to *bla*CMY42 (HM146927.1). The last variant is similar to CTX131 (NG_048801.1), which contains four different mutations, as indicated by red arrow in figure 4A. A phylogenetic tree clarified the similarities and differences between local *bla*CMY genes and many international gene sequences obtained from NCBI blast searches and GenBank (Fig. 4B).

Discussion

Microorganisms are the most common cause of urinary tract infections. Pathogenic bacteria invade the urinary tract through the urethra mostly and then they ascend to the urinary bladder and kidneys to start multiplication and development of infection [39]. *E. coli* is the main reason for UTIs compared to other microorganisms and it is estimated to constitute 50% and 80% of nosocomial and community acquired urinary tract infection cases, respectively [9,40]. Many factors have a role in developing UTIs and may affect the rate of it, including gender, age, using catheters, and immunity [7]. All patients who were directed to this bacteriological laboratory had problems and clinical

symptoms in the urinary tract. In this study, 35.9% of the urinary tract infections were caused by bacteria including mixed bacterial infections. The rate of infection was higher in females, 67.1%, than in males, 32.8%. Abnormal samples of urine containing pus were observed in the urine of these patients who have bacterial infection before sending them to further bacteriological examination by specialist. This finding is comparable with other studies and it is presumably due to anatomical composition differences of urethra in females [41,42]. Gram negative bacteria constituted the highest rate of infection, 98%, among bacterial causative agents, while gram positive bacteria were recovered in only 19.1%. *E. coli* constituted 32 (15.7%) of UTI patients and 43.8% of gram negative bacteria isolates. This high rate of *E. coli* isolated from urine samples of patients prove that *E. coli* was the major cause of UTI and this study agrees with other studies that *E. coli* is observed as a common source of UTIs [9,43].

In this study, *E. coli* was tested for two existing marker genes, *uid*A and *usp*A. These two genes together showed the firm confirmation of *E. coli*. All isolates showed a positive PCR amplification for both genes using a specific set of primers for flanking regions of *usp*A [27] and specific primers for *uid*A [26]. It was concluded before that *uid*A is not very accurate alone because it detects not only *E. coli*, but also some *Shigella* species [26,44]. So using two genes instead of one decrease the cross reactivity of the *uid*A primer with *Shigella* species [34]. All *E. coli* isolates were subjected to phylogenetic grouping analysis and classification of *E. coli* isolates on four groups, A, B1, B2, and D. Based on the existence of one or more genes of the chuA, yjaA and DNA fragment TSPE4.C2, *E. coli* is classified under three phylogenetic groups A, B1, B2, and D. Most *E. coli* isolates, 37.5% and 34.3%, were found under group B2 and D, respectively. Most *E. coli* isolates having β-lactamase resistance genes were also discovered in these two *E. coli* phylogenetic groups (Tab. 3), which are clinically important because most pathogenic *E. coli* and extra intestinal isolates come from these phylogenetic groups [45]. While both groups A and B1 recorded only in 28.1% of the isolates together.

Antibiotics are the most common and important antimicrobial agents used to treat urinary tract infections caused by bacteria. Appearance of drug resistance *E. coli* is increasing nowadays and causes difficulty in treatment and poor responses to antibiotic therapy [13]. The existence of multi-drug resistance *E. coli* is a serious problem and it makes a major public health concern. Bacteria bearing β-lactamase resistance genes have the ability to destroy different β-lactam antibiotics and impede treatment. Recently, complicated UTI cases have been difficult to manage and control due to the appearance of *E. coli* producing extended spectrum β-lactamases (ESBL), which causes multi-drug resistance [8,12,14–16]. Therefore, antibiotic sensitivity test of bacteria against different antibiotics is necessary, and finding the prevalence of resistance genes is significantly important. Our study showed that 100% of *E. coli* isolates were resistant to many antibiotics, including tetracycline, rifampin. Gentamycin and the resistance rate against trimethoprim, cepahalothin was also high, with the rate of 96.8%, and 93.7%, respectively. On the other hand, some antibiotics still showed affective action against *E. coli* and it can be used as an antibiotic of choice for treatments, including imepenem and meropenem. All patients who had a bacterial infection were put on an antibiotic course of treatment and the common affective used antibiotics were imepenem, amikacin, ciprofloxacin, and nitrofurantoin after antibiotic sensitivity testing. This result is alarming and is clinically important because these antibiotics are known as the first antibiotic of choice for *E. coli* infection treatment in humans [17]. In addition, 95% of the isolates showed multi-drug resistance against most of the antibiotics and two isolates were resistant to all antibiotics tested in this study. Three female patients have complicated cases. They do not respond to antibiotic treatment. Two of them have been repetitively visiting the hospital for more than a year for a recurrent infection and failing of antibiotic treatment. Multi-drug resistant *E. coli* was detected previously in different studies in different countries, which are comparable with our study [30,46–49].

All the isolates recovered from urine of the patients with UTIs were tested for detection of different β-lactamase resistance genes using PCR. *bla*CTX is responsible for developing drug resistance against the new generation of β-lactam antibiotic, cefotaxime (CTX) [20–23], so in this study, an attempt was made to find the prevalence of *bla*CTX in *E. coli* isolates. Positive amplification of *bla*CTX resistance gene was observed with 84.3% of the isolates. All bacteria harboring this gene were found to show resistance to cefotaxime. This finding

agrees with different studies in different countries globally that *bla*CTX is the most common ESBLs in *E. coli* [50–53]. The second most common antibiotic resistance gene found in this study was *bla*TEM, 28.1%. Existence of *bla*TEM is responsible for resisting the action of amoxicillin-clavulanic and ampicillin [17,54]. This result showed similar result with previous studies that *bla*CTX is the most common among *E. coli* and followed by *bla*TEM [55–58]. At the same time, *bla*OXA was observed in the same rate as *bla*TEM, 28.1%, which is needed to develop resistance to oxacillin and coxacillin. It is very poorly inhibited by clavulanic acid in bacteria [17,59], but *bla*CMY was detected in less numbers of isolates by 15.6%. Meanwhile, all the recovered isolates were negative for *bla*SHV. The current study showed that the prevalence of β-lactamase resistance gene is very high and reached alarming levels. Existing *E. coli* bacteria showing multi-drug resistances is very serious and of clinical importance. So, very serious and well-regulated plans are required to control, manage, and stop the development and spread of resistance bacteria among patients, especially in hospitals.

Sequence analysis of *bla*OXA and *bla*CMY confirmed the correct PCR amplified genes. All seven blaOXA gene sequences esembled each other without having any mutations, but *bla*CMY had different variants with different polymorphism sequences which was revealed through multisequence alignments. These variants, CMY2 (CP043945.1), CMY42 (HM146927.1), and CMY131 (NG_048801.1), were previously discovered and published in different countries, but interestingly three different variants were found in a single location of this study in a small population. This means that this gene has many variants and most of them may exist in the study area, which makes a diversity of *E. coli* bacteria resistant to beta lactam antibiotics. These mutations of these variants were clearly clarified via multiplesequence alignment of the recovered genes (Fig. 4A) and the drawn phylogenetic tree showed the differences clearly between variants (Fig. 4B).

This study has concluded that *E. coli* is the most common cause of urinary tract infection, and it can become complete resistant against many antibacterial agents, including extended spectrum β-lactam antibiotics (ESBLs) such as tetracycline, rifampin, gentamycin, doxycycline, trimethoprim, and cepahalothin. It has also showed the emergence of *E. coli* containing extended spectrum β-lactamase resistance genes among hospitalized patients in Teaching hospital in Sulaimany city, which is a very serious health concern. Majority of the infectious *E. coli* in this study was under phylogenetic group B2 and D. Different types of ESBLs exist among *E. coli* isolates including *bla*CTX, *bla*OXA, *bla*CMY2 and *bla*TEM. *bla*CTX was the most common ESBL among *E. coli,* reaching alarming levels. Regular surveillance is necessary to detect ESBLs among *E. coli* isolates and even in other types of pathogen bacteria in the area of the study. Therefore, strict control polices by authority and health sectors are a need to tackle the problem of antibiotic resistance. In addition, it is important to avoid unnecessarily using antibiotics without physician prescriptions. For every bacterial infection, it is recommended to carry out antibiotic sensitivity test before antibiotic prescription.

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References

[1] Murugan K., Savitha T., Vasanthi S. 2012. Retrospective study of antibiotic resistance among uropathogens from rural teaching hospital, Tamilnadu, India. *Asian Pacific Journal Tropical Disseases* 2: 375–380. doi:10.1016/S2222-1808(12)60082-6

[2] Chakupurakal R., Ahmed M., Sobithadevi D.N., Chinnappan S., Reynolds T. 2010. Urinary tract pathogens and resistance pattern. *Journal of Clinical Pathology* 63: 652–654. doi:10.1136/jcp.2009.074617

[3] Karlowsky J.A., Lagacé-Wiens P.RS., Simner P.J., DeCorby M.R., Adam H.J., Walkty A., Hoban D.J., Zhanel G.G. 2011. Antimicrobial resistance in urinary tract pathogens in Canada from 2007 to 2009: CANWARD surveillance study. *Antimicrobial Agents Chemotherapy* 55: 3169–3175. doi:10.1128/AAC.00066-11

[4] Guion C.E., Ochoa T.J., Walker C.M., Barletta F., Cleary T.G. 2008. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *Journal of Clinical Microbiology* 46: 1752–1757.

doi:10.1128/JCM.02341-07
[5] Akram M., Shahid M., Khan A.U. 2007. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials* 6: 1–7. doi:10.1186/1476-0711-6-4
[6] Chomarat M. 2000. Resistance of bacteria in urinary tract infections. *International Journal of Antimicrobial Agents* 16: 483–487. doi:10.1016/S0924-8579(00)00281-8
[7] Igbal T., Naqvi R., Akhter S. 2011. Frequency of UTI in renal transplant recipient and effect on graft function. *Journal of Paksitan Medical Association* 60: 826–829.
[8] Vaidya V. 2011. Horizontal transfer of antimicrobial resistance by extended-spectrum β-lactamase-producing Enterobacteriaceae. *Journal of Laboratory Physicians* 3: 37–42. doi:10.4103/0974-2727.78563
[9] Ramanath K.V., Shafiya S.B. 2011. Prescription pattern of antibiotic usage for urinary tract infection treated in a rural tertiary care hospital. *Indian Journal of Pharmacy Practices* 4: 57–63.
[10] Ejrnæs K. 2011. Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*. *Danish Medical Bulletin* 58: article number B4187.
[11] Lindsay E., Nicolle M.D. 2002. Epidemiology of urinary tract infections. *Clinical Microbiology Newsletter* 24: 135–140. doi:10.1016/S0196-4399(02)80035-6
[12] Mukherjee M., Basu S., MuKherjee SK.M., Majumder M. 2013. Multidrug-resistance and extended spectrum beta-lactamase production in uropathogenic *E. coli* which were isolated from hospitalized patients in Kolkata, India. *Journal of Clinical Diagnostic Research* **7**: 449–453. doi:10.7860/JCDR/2013/4990.2796
[13] Grover N., Sahni AK., Bhattacharya S. 2013. Therapeutic challenges of ESBLS and Ampc beta-lactamase producers in a tertiary care center. *Medical Journal of Armed Forces India* 69: 4–10. doi:10.1016/j.mjafi.2012.02.001
[14] Khanna N., Boyes J., Lansdell P.M., Hamouda A., Amyes SG.B. 2012. Molecular epidemiology and antimicrobial resistance pattern of extended-spectrum-β-lactamase-producing Enterobacteriaceae in Glasgow, Scotland. *Journal of Antimicrobial Chemotherapy* 67: 573–577. doi:10.1093/jac/dkr523
[15] Xiao Y.H., Giske C.G., Wei Z.Q., Shen P., Heddini A., Li L.J. 2011. Epidemiology and characteristics of antimicrobial resistance in China. *Drug Resististance Updates* 14: 236–250. doi:10.1016/j.drup.2011.07.001
[16] Zhang Y., Yang J., Ye L., Luo Y., Wang W., Zhou W., Cui Z., Han L. 2012. Characterization of clinical multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates, 2007–2009, China. *Microbial Drug Resistance* 18: 465–470. doi:10.1089/mdr.2012.0016
[17] Bradford P.A. 2001. Extended-spectrum-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical Microbiology Reviews* 14: 933–951. doi:10.1128/CMR.14.4.933-951.2001
[18] Paterson D.L., Bonomo R.A. 2005. Extended spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews* 18Ł 657–686. doi:10.1128/CMR.18.4.657-686.2005
[19] Lakshmi R., Nusrin K.S., Georgy S.A., Sreelakshmi K.S. 2014. Role of beta-lactamases in antibiotic resistance: a review. *International Research Journal of Pharmacy* 5: 37–40. doi:10.7897/2230-8407.050207
[20] Barthélémy M., Péduzzi J., Bernard H., Tancrède C., Labia R. 1992. Close amino acid sequence relationship between the new and chromosomally encoded enzymes of *Klebsiella oxytoca*. *Biochimica et Biophysica* 1122: 15–22. doi;10.1016/0167-4838(92)90121-S
[21] Madhumati B., Rani L., Ranjini C.Y., Rajendran R. 2015. Prevalence of AMPC beta lactamases among gram negative bacterial isolates in a Tertiary Care Hospital. *International Journal of Current Microbiology and Applied Sciences* 4: 219–227.
[22] Bauernfeind A., Casellas J.M., Goldberg M., Holley M., Jungwirth R., Mangold P., Rohnisch T., Schweighart S., Wilhelm R. 1992. A new plasmidic cefotaximase from patients infected with *Salmonella typhimurium*. *Infection* 20: 158–163. doi:10.1007/BF01704610
[23] Dutour C., Bonnet R., Marchandin H., Boyer M., Chanal C., Sirot D., Sirot J. 2002. CTX-M-1, CTX-M-3, and CTX-M-14 β-lactamases from Enterobacteriaceae isolated in France. *Antimicrobial Agents and Chemotherapy* 46: 534–537. doi:10.1128/AAC.46.2.534-537.2002
[24] Cockerill F.R. 1999. Genetic methods for assessing antimicrobial resistance. *Antimicrobial Agents and Chemotherapy* 43: 199–212. doi:10.1128/AAC.43.2.199
[25] Harley J.P., Prescott L.M. 2002. Laboratory exercises in microbiology. Boston, MA, McGraw-Hill Publishers. http://125.212.201.8:008/handle/DHKTYTHD_123/2722
[26] Heijnen L. Medema G. 2006. Quantitative detection of *E. coli*, *E. coli* O157 and other toxin producing *E. coli* in water samples using a culture method combined with real-time PCR. *Journal of Water Health* 4: 487–498. doi:10.2166/wh.2006.0032
[27] Chen J., Griffiths M.W. 1998. PCR differentiation of *Escherichia coli* from other gram-negative bacteria using primers derived from the nucleotide sequences flanking the gene encoding the universal stress

protein. *Letter in Appllied Microbiology* 27: 369–371. doi:10.1046/j.1472-765X.1998.00445.x

[28] Bonacorsi P., Clermont O., Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology* 66: 4555–4558. doi:10.1128/AEM.66.10.4555-4558.2000

[29] Ahmed A.M., Motoi Y., Sato M., Maruyama A., Watanabe H., Fukumoto Y., Shimamoto T. 2007. Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes. *Applied and Environmental Microbiology* 73: 6686–6690. doi:10.1128/AEM.01054-07

[30] Ahmed A.M., Shimabukuro H., Shimamoto T. 2009. Isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in Japan. *Journal of Food Science* 74: 405–410. doi:10.1111/j.1750-3841.2009.01291.x

[31] Benson D. A., Clark K., Karsch-Mizrachi I., Lipman D.J., Ostell J., Sayers E.W. 2015. GenBank. *Nucleic Acids Research* 43 (database issue): D30–D35. doi:10.1093/nar/gku1216.

[32] Dereeper A., Audic S., Claverie JM., Blanc G. 2010. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evolutionary Biology* 10: 8–13. doi:10.1186/1471-2148-10-8

[33] Clinical and Laboratory Standards Institute. 2012. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement M100-S22 32. http://www.antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf

[34] Godambe L.P., Bandekar J., Shashidhar R. 2017. Species specific PCR based detection of *Escherichia coli* from Indian foods. *3 Biotech* 7: 1–5. doi:10.1007/s13205-017-0784-8

[35] Tozzi A.E., Caprioli A., Minelli F., Gianviti A., De Petris L., Edefonti A., Montini G., Ferretti A., De Palo T., Gaido M., Rizzoni G., Bettinelli A., Capasso G., Caringella A., Coppo R., Lama G., Li Volti S., Maffei S., Maringhini S., Miglietti N., Pecoraro C., Pela I., Pennesi M., Penza R., Peratoner L., Perfumo F., Ratsche I., Salvaggio E., Setzu C., Zacchello G. 2003. Shiga toxin-producing *Escherichia coli* infections associated with hemolytic uremic syndrome, Italy, 1988-2000. *Emerging Infectious Diseases* 9: 106–108. doi:10.3201/ eid0901.020266

[36] Moussa I.M., Ashgan M.H., Alwathnani H.A., Mohamed K.F., Al-Doss A.A. 2010. Multiplex polymerase chain reaction for detection and characterization of shiga toxigenic *Escherichia coli* (STEC). *African Journal of Biotechnology* 9: 4356–4363. http://www.academicjournals.org/AJB

[37] Kuiper E.J., Soonawala D., Vermont C., van Dissel J.T. 2011. Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* o104:H4 in the Netherlands, May 2011. *Eurosurveillance* 16: 2–4. doi:10.2807/ese.16.25.19897-en

[38] Guion C.E., Ochoa T.J., Walker C.M., Barletta F., Cleary T.G. 2008. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *Journal of Clinical Microbiology* 46: 1752–1757. doi:10.1128/JCM.02341-07

[39] Boye A., Siakwa P., Boampong J., Koffuor G., Ephraim R., Amoateng P., Obodai G., Penu D. 2012. Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. *E3 Journal of Medical Research* 1: 74–83. http://www.e3journals.org

[40] Kot B. 2019. Antibiotic resistance among uropathogenic *Escherichia coli*. *Polish Journal of Microbiology* 68: 403–415. doi:10.33073/pjm-2019-048

[41] Zeyaullah M., Kaul V. 2015. Prevalence of urinary tract infection and antibiotic resistance pattern in Saudi Arabia population. *Global Journal of Biology, Agriculture and Health Sciences* 4: 206–214. http://www.gifre.org

[42] Hellerstein S. 1998. Urinary tract infections in children: why they occur and how to prevent them. *American Family Physician* 57: 2440–2446. https://www.aafp.org/afp/1998/0515/p2440.html

[43] Michno M., Sydor A., Wałaszek M., Sułowicz W. 2018. Microbiology and drug resistance of pathogens in patients hospitalized at the nephrology department in the South of Poland. *Polish Journal of Microbiology* 67: 517–524. doi:10.21307/pjm-2018-061

[44] Bej A.K., DiCesare J.L., Haff L., Atlas R.M. 1991. Detection of *Escherichia coli* and *Shigella* spp. in water by using the polymerase chain reaction and gene probes for uid. *Applied and Environmental Microbiology* 57: 1013–1017. doi:10.1128/aem.57.4.1013-1017.1991

[45] Bingen E., Picard B., Brahimi N., Mathy S., Desjardins P., Elion J., Denamur E. 1998. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. *Journal of Infectious Diseases* 177: 642–650. doi:10.1086/514217

[46] Sunde M. 2005. Prevalence and characterization of class 1 and class 2 integrons in *Escherichia coli* isolated from meat and meat products of Norwegian origin. *Journal of Antimicrobial Chemotherapy* 56: 1019–1024. doi:10.1093/jac/dki377

[47] Yan J.J., Hong C.Y., Ko W.C., Chen Y.J., Tsai S.H., Chuang C.L., Wu J.J. 2004. Dissemination of blaCMY-2 among *Escherichia coli* isolates from food animals, retail ground meats, and humans in Southern Taiwan. *Antimicrobial Agents and Chemotherapy* 48: 1353–1356. doi:10.1128/AAC.48.4.1353-1356.2004

[48] Hansen T.B., Jensen T.I., Clausen B.H., Bramsen J.B., Finsen B., Damgaard C.K., Kjems J. 2013. Natural RNA circles function as efficient microRNA sponges. *Nature* 495: 384–388. doi:10.1038/nature11993

[49] Forward K.R., Matheson K.M., Hiltz M., Musgrave H., Poppe C. 2004. Recovery of cephalosporin-resistant *Escherichia coli* and *Salmonella* from pork, beef and chicken marketed in Nova Scotia. *Canadian Journal of Infectious Diseases* 15: 226–230. doi:10.1155/2004/695305

[50] Zhao W.H., Hu Z.Q. 2013. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. *Critical Reviews in Microbiology* 39: 79–101. doi:10.3109/1040841X.2012.691460

[51] Cantón R., Coque T.M. 2006. The CTX-M β-lactamase pandemic. *Current Opinion in Microbiology* 9: 466–475. doi:10.1016/j.mib.2006.08.011

[52] Rossolini G.M., Andrea M.M.D., Mugnaioli C. 2008. The spread of CTX-M-type extended-spectrum β-lactamases. *Clinical Microbiology of Infection* 14: 33–41. doi:10.1111/j.1469-0691.2007.01867.x

[53] Zeynudin A., Pritsch M., Schubert S., Messerer M., Liegl G., Hoelscher M., Belachew T., Wieser A. 2018. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. *BMC Infectious Disseases* 18: 1–10. doi:10.1186/s12879-018-3436-7

[54] Taylor N.M., Davies R.H., Ridley A., Clouting C., Wales A.D., Clifton-Hadley F.A. 2008. A survey of fluoroquinolone resistance in *Escherichia coli* and thermophilic *Campylobacter* spp. on poultry and pig farms in Great Britain. *Journal of Applied Microbiology* 105: 1421–1431. doi:10.1186/s12879-018-3436-7

[55] Naseer U., Sundsfjord A. 2011. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. *Microbial Drug Resistance* 17: 83–97. doi:10.1089/mdr.2010.0132

[56] Livermore D.M., Canton R., Gniadkowski M., Nordmann P., Rossolini G.M., Arlet G., Ayala J., Coque T.M., Kern-Zdanowicz I., Luzzaro F., Poirel L., Woodford N. 2007. CTX-M: changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy* 59: 165–174. doi:10.1093/jac/dkl483

[57] Peirano G., Pitout JD.D. 2010. Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4. *International Journal of Antimicrobial Agents* 35: 316–321. doi:10.1016/j.ijantimicag.2009.11.003

[58] Adwan K., Jarrar N., Abu-Hijleh A., Adwan G., Awwad E. 2014. Molecular characterization of *Escherichia coli* isolates from patients with urinary tract infections in Palestine. *Journal of Medical Microbiology* 63: 229–234. doi:10.1099/jmm.0.067140-0

[59] Bush K., Jacoby G.A., Medeiros A.A. 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy* 39: 1211–1233. doi:10.1128/AAC.39.6.1211

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