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Prevalence of Some Group A Beta-Lactamase Genes among Uropathogenic *Escherichia coli* Isolated from Women with Cystitis

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ABSTRACT

Urinary tract infection is a common infection associated with considerable societal cost and even increasing antibiotic resistance, which to some extent represents a challenging issue facing infection control. In this work, some group A Beta-lactamase genes bla_{TEM}, bla_{SHV}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, $bla_{CTX-M-25}$ among Uropathogenic *Escherichia coli* from women with cystitis have been detected. The results showed that 100 isolates of 611 urine samples belonged to *Escherichia coli*. Antibiotic susceptibility testing of 100 isolates to 14 antibiotics revealed that 63%, 58%, 36%, 27%, 14%, 6%, 4%, 30%, 26%, 4%, 16%, 2%, and 44% of the isolates were resistant to Ceftazidime, Cefotaxime, Piperacillin, Amoxicillin-clavulanate, Aztreonam, Piperacillin-tazobactam, Imipenem, Meropenem, Levofloxacin, Ciprofloxacin, Gentamicin, Amikacin, Nitrofurantoin, and Trimethoprome-sulfamethoxazole, respectively. The results revealed that 29% of isolates were multidrug resistant. In the current study, the results of molecular detection showed the predominance of ESBL genes in *Escherichia coli* isolates: bla_{TEM} 98% followed by bla_{SHV} 69%, and then, $bla_{CTX-M-1}$ 66%. $bla_{CTX-M-9}$ only appeared in one isolate. Both $bla_{CTX-M-2}$ and $bla_{CTX-M-25}$ were not detected. The study concludes the high spreading of coexistence of more than one gene of Group A β-lactamase genes among uropathogenic *Escherichia coli* causes them to resist many antibiotics. This makes the treatment regimen unusual or hard to be achieved.

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Introduction

Urinary tract infection (UTI) is one of the most common microbial infections worldwide, with a global prevalence of 10 per 1,000 people. UTI is associated with considerable societal cost, significant morbidity, and even increasing antibiotic resistance, which represents a current challenge for infection control (1, 2). Half of all women suffer from UTI at least once in their life, which is a common bacterial infection (3). Most UTIs in women are episodes of uncomplicated acute cystitis that occur in women of childbearing age (4). Although acute uncomplicated cystitis may not be thought of as a serious condition, it affects the patient's quality of life by causing an estimated six days of discomfort (5). Complicated urinary tract infections can happen, either in the upper or lower urinary tract, but are accompanied by an underlying condition that increases the risk of treatment failures, such as obstruction, anatomical malformation, urinary tract abnormality, pregnancy, or resistant pathogens (5). *Escherichia coli*, from the Enterobacteriaceae family, is the most frequent bacterial agent in both nosocomial and community-acquired UTIs. About 70–80% of all uropathogen is *E. coli* (6). *E. coli* is a Gram- negative commensal of the distal colon which also harbors other anaerobic bacteria, including Bacteroides and Bifidobacteria (7). B-Lactam antibiotics due to their broad antibacterial spectrum and minimal side effects are widely used in the treatment of various infections such as UTI (8). According to Ambler classification, BLEs are grouped in serine-BLEs (SBLEs) of class A, C, and D, and metal-BLEs (MBLEs) of class B (9). Extended-spectrum beta-lactamases (ESBLs) are the group of beta-lactamase enzymes, which hydrolyze and cause resistance to the oxyimino-cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) and monobactams (aztreonam), but not the cephamycins (cefoxitin and cefotetan) or carbapenems (imipenem, meropenem, and ertapenem), produced by *Escherichia coli* and *Klebsiella pneumoniae* (10). Many different types of ESBLs have been described until now. However, the most common ones are derivatives of the SHV, TEM and CTX-M enzymes (11). The CTX-M types have different 50 types divided into 5 groups according to their amino acids CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (12). Currently, more than 140 different types of TEM-type beta-lactamases are known, TEM-1 being most encountered in *E. coli* and *K. pneumoniae*. There are also more than 100 different types of SHV, especially existent in *Pseudomonas aeruginosa* and *Acinetobacter* spp. (13). The current work aims to investigate some group A Beta-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-9}$, $bla_{CTX-M-25}$) among Uropathogenic *Escherichia coli* from women with cystitis.

Materials and Methods

Collection of urine samples and Bacterial identification

A total of 611 urine samples were collected from wom-

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en suffering from cystitis from different hospitals in Al-Hilla city, Babylon province, Iraq, during a period from May 2021 to December 2021. The urine samples were collected in sterile screw-capped test tubes. Microscopic examination was conducted to ensure the presence of White blood cells in urine samples and after centrifugation, the precipitate was directly streaked on MacConkey agar and Eosin Methylene blue agar. The samples were incubated at 37°C for 24 hours. Additionally, the lactose fermenter Green metallic sheen isolates of *E. coli* were confirmed by PCR amplification using primer pairs specific for *E. coli* 16S rRNA gene.

Research Methods

Antimicrobial Susceptibility Testing

Antibacterial susceptibility of *E. coli* isolates against 14 antibiotic agents was investigated. The antimicrobial agents β-lactams (amoxicillin-clavulanic acid 20/10 µg; cefotaxime 30 µg, ceftazidime 30 µg, aztreonam 30 µg, imipenem 10 µg, meropenem 10 µg, Piperacillin 100 µg, Piperacillin-tazobactam100/10 µg), sulfonamides (trimethoprim-sulfamethoxazole 1.25/23.75 µg), Nitrofurantoin 300 µg, quinolones (ciprofloxacin 5 µg and Levofloxacin 5 μg), and aminoglycosides (gentamicin 10 µg and amikacin 30 µg) were tested and determined via disc diffusion method according to clinical and laboratory standards institute CLSI-2021, (14). Activation of isolates was performed using brain heart brothel at 37°C for 18 hrs and the growth was adjusted to 0.5 MacFarland's standard (1.5×10^8 C F U / mL). Then the isolates were spread on Mueller Hinton agar (MHA) with a sterile cotton swab. Antibiotic disks were placed onto MHA, gently pressed down to ensure complete contact with the agar inoculated with bacteria, incubated for 18-20 hr at 37°C, and then the inhibition zone diameter in millimeters (mm) was recorded to interpret whether the isolates were sensitive or resistant according to CLSI-2021 (14).

Genomic DNA extraction and purification

E. coli genomic DNA was extracted from isolates and purified using DNA extraction kits (FavorPrep Genomic DNA Mini Kit Favorgen, Taiwan) according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

The composition of the reaction mixture was 12.5 µl of master mix (Promega, USA), 1.5µl of both forward and reverse primers, and 6.5 µl PCR water. 3µl of extracted DNA was transferred with a reaction mixture. Gene amplification of *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M-1}$, *bla* $_{CTX-M-2}$, *bla* $_{CTX-M-9}$, and bla $_{CTX-M-25}$ was carried out in thermocycler according to the temperature cycles listed in table 1.

Gel electrophoresis

The amplified products were analyzed by gel electrophoresis. 1.5% Agarose gel was prepared in Tris/borate/EDTA (TBE) buffer, 4µl of ethidium bromide dye was added to the solution and solidified in gel casting assem-

Table 1. The specific primers and PCR condition.

Primer name	5 to 3 sequence	Product size (bp)	Conduction	Reference
bla $_{TEM}$	F-CGTGTCGCCCTTATTCCCT R-GCAACTTTATCCGCCTCCAT	626	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 58.1ºC, 30 sec. Step 4: 72ºC, 70.0 sec. Step 5: 72ºC, 5 min.	
bla $_{SHV}$	F- TCTGGTGGACTACTCGCC R-TCGTCCACCATCCACTGC	341	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 58.9ºC, 30 sec. Step 4: 72ºC, 40.0 sec. Step 5: 72ºC, 5 min.	
bla $_{CTX-M-1}$	F-CAGTCCACGACGTCGGTAA R-CAGTTCACGCTGATGGCGAC	462	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 62.4ºC, 30 sec. Step 4: 72ºC, 50.0 sec. Step 5: 72ºC, 5 min.	
bla $_{CTX-M-2}$	F-CCTGCTATTTAGCAGCG R-AGGTCGCTCTTCTTGATT	261	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 52.4ºC, 30 sec. Step 4: 72ºC, 30.0 sec. Step 5: 72ºC, 5 min.	
bla $_{CTX-M-9}$	F-GAGAGTGCAACGGATGATGTT R-CAGTCCACGACGTCGGTAA	689	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 58.3ºC, 30 sec. Step 4: 72ºC, 70.0 sec. Step 5: 72ºC, 5 min.	
bla $_{CTX-M-25}$	F-TAATGACGACAGCCTGTGTTTC R-CGCTCAACTCCCCGAATGT	348	Step 1: 95ºC, 2 min. Step 2: 95ºC, 30 sec. Step 3: 58.5ºC, 30 sec. Step 4: 72ºC, 40.0 sec. Step 5: 72ºC, 5 min.	(15)
16S rRNA	F- GAAGCTTGCTTCTTTGCT R- GAGCCCGGGGATTTCACAT	541	Step 1:95 C, 5 min Step 2:94 C , 45 sec Step 3:55 C , 45 sec Step 4:72 C ,1.5 min Step 5: 72 c ,5 min	Sabat *et al* (16).

bly. The amplified products and 100bp DNA ladder were loaded on the gel and attached to electrodes of a potential difference of (80-100 volts) for 80 minutes. The amplified genes were compared with the DNA ladder under a UV transilluminator.

Results

Bacterial identification and Antimicrobial Sensitivity testing

Out of 611 urine samples of UTI, the results showed that 100 isolates belonged to *Escherichia coli*, which appeared in pink color on MaConkey agar and Green metallic sheen on Eosin Methylene blue. The isolates were also identified by the specific gene 16S rRNA 541 bp. (Fig. 1).

Antibiotic susceptibility testing of 100 *E. coli* isolates to 14 antibiotics was performed on Muller-Hinton agar using the disc diffusion method. The Antimicrobial susceptibility in Figure 2 showed an increase in resistance levels for most of the beta-lactam that was resistant to ceftazidime at 63% followed by cefotaxime and piperacillin at 58%, amoxicillin-clavulanate at 36%, aztreonam of 27%, and piperacillin-tazobactam of 14%. The results showed that 26% of *E.coli* isolates were resistant to ciprofloxacin, 30% to levofloxacin, and 44% to trimethoprim-sulfamethoxazole). This study also showed low resistance to imipenem, meropenem, and nitrofurantoin of 6%, 4%, and 2%, respectively. *E. coli* isolates in the study revealed low resistance of 16% to gentamicin and 4% to amikacin. In addition, 29% of isolates are multidrug-resistant (MDR) to antibiotics.

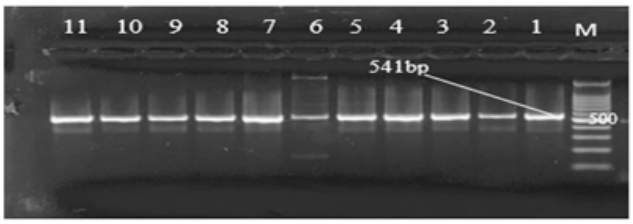

Figure 1. 1.5% Agarose gel electrophoresis (in TBE) of 16S rRNA gene (541 bp) amplicon. M lane, represents the 100bp ladder and the rest lanes represent the samples.

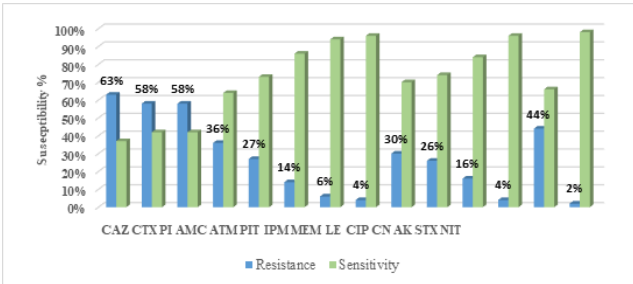

Figure 2. Antimicrobial susceptibility of *E. coli* isolates (Ceftazidime CAZ, Cefotaxime CTX, Piperacillin PI, Amoxicillin-clavulanate AMC, Aztreonam ATM, Piperacillin-tazobactam PIT, Imipenem IMP, meropenem MEM, Levofloxacin LE, Ciprofloxacin CIP, Gentamycin CN, Amikacin AK, trimethoprim-sulfamethoxazole STX, and Nitrofurantoin NIT).

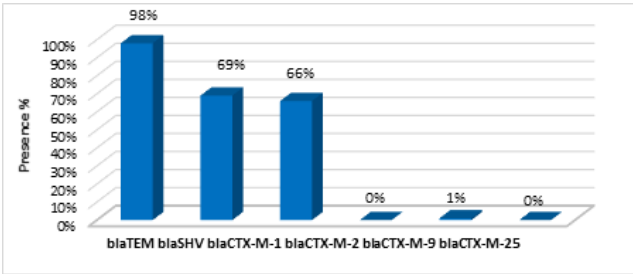

Figure 3. Distribution of *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M-1}$, *bla* $_{CTX-M-2}$, *bla* $_{CTX-M-9}$, *bla* $_{CTX-M-25}$ genes with their percentages among *E. coli* isolates.

Detection of Beta-lactamase genes *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M-1}$, *bla* $_{CTX-M-2}$, *bla* $_{CTX-M-9}$, *bla* $_{CTX-M-25}$, among Isolates of *E.coli*

The results of the detection of beta-lactamase genes, *bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M-1}$, *bla* $_{CTX-M-2}$, *bla* $_{CTX-M-9}$, *bla* $_{CTX-M-25}$ in 100 isolates of *E.coli* were found as 98%, 69 %66%, 0%, 1%.0%, respectively as shown in Figure 3.

The results showed the predominant ESBL gene in E. coli isolates was TEM (Fig. 4) followed by bla $_{SHV}$ (Fig. 5), and then, bla $_{CTX-M-1}$ (Fig. 6). Bla $_{CTX-M-9}$ only appeared in one isolate, while bla $_{CTX-M-2}$ and bla $_{CTX-M-25}$ were not found in any isolate.

Coexistence of β-lactamase genes

The present study showed that there was the coexistence of beta-lactamase genes in the same isolates. The results revealed that 67 of the *E.coli* isolates harbored (*bla* $_{TEM}$, *bla* $_{SHV}$) genes, 65 isolates harbored (*bla* $_{TEM}$, *bla* $_{CTX-M-1}$), 43 isolates harbored (*bla* $_{SHV}$, *bla* $_{CTX-M-1}$) genes, and 42 isolates harbored (*bla* $_{TEM}$, *bla* $_{SHV}$, *bla* $_{CTX-M-1}$). Table 2 shows the distribution of coexistence genes among *E.coli*

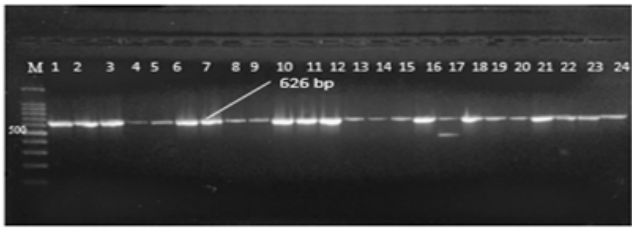

Figure 4. 1.5% Agarose gel electrophoresis (in TBE) of *bla* $_{TEM}$ gene at (626bp) amplicon. M lane, represents the 100bp ladder and the rest lanes represent the samples.

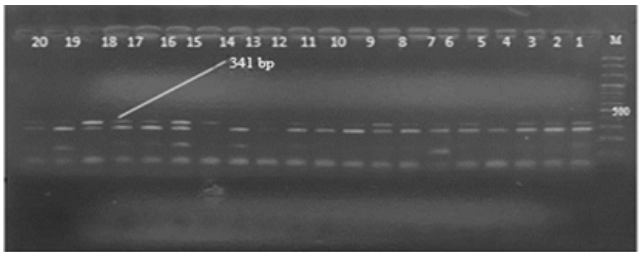

Figure 5. 1.5% Agarose gel electrophoresis (in TBE) of *bla* $_{SHV}$ gene at (341bp) amplicon. M lane represents the 100bp ladder and the rest lanes represent the samples.

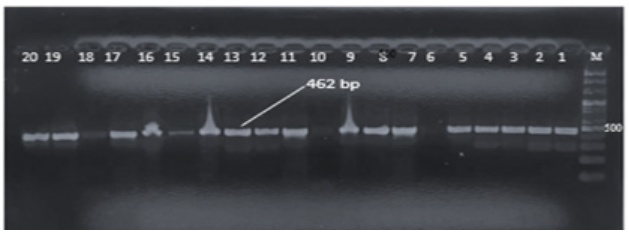

Figure 6. 1.5% Agarose gel electrophoresis (in TBE) of *bla* $_{CTX-M-1}$ *gene* at (462bp) amplicon. M lane represents the 100bp ladder and the rest lanes represent the samples.

Table 2. Coexistence group A Beta-Lactamase genes.

β-lactamases genes	No. (%)
bla $_{TEM}$ bla $_{SHV}$	67 (67%)
bla $_{TEM}$ bla $_{CTX-M-1}$	65 (65%)
bla $_{SHV}$ bla $_{CTX-M-1}$	43 (43%)
bla $_{TEM}$ bla $_{SHV}$ bla $_{CTX-M-1}$	42 (42%)

isolates.

Discussion

Urinary tract infection is a very common disease among females, and its diagnosis and treatment have important implications for patient health, healthcare costs, and the development of antibiotic resistance (17). Prevalence studies on local urinary tract pathogens and their susceptibility patterns to antimicrobial agents are useful to guide experimental antibiotic therapy because the prevalence and characteristics of urinary pathogens can vary with time and geographical regions (18). As previous studies have shown, *Escherichia coli* can be found in the bladder of women with or without symptoms of a UTI (19, 20). In Dohuk city, Iraq, a significantly higher rate of UPEC infection was reported which was 74.4% (276/371) among women (21). In another study conducted in Turkey on 429 women between the ages of 18 to 65 years, *E. coli* is found to be the most prevalent causative agent of UTI (22).

Previous studies approached the resistance of *E. coli* to antibiotics like that of Jalil and Al Atbee (23) which showed that *E. coli* isolates were found to be highly resistant to cefotaxime (79.3%), aztreonam (74.4%), and ceftazidime (68.3%). The reasons for high resistance percentages of *E. coli* isolates to βlactam antibiotics may be due to the wide and wise-less use of antibiotics, which is leading to the development of resistance by the action of β-lactamase enzymes, which may be either chromosomally or plasmid-mediated. Resistance to 3rd generation of cephalosporines was mainly due to the ESBL enzyme that can hydrolyze 3rd generation cephalosporines and aztreonam antibiotics (24).

The increasing emergence of resistant *Escherichia coli* to Fluoroquinolones has been reported worldwide and this is likely due to the excessive use of these antibiotics (25). UPEC resistance to Fluoroquinolones from various countries has been reported the resistance level is significant (25). Prasada *et al*. (26) reported a high incidence of fluoroquinolone resistance in UPEC in India (>60%). A study (27) showed that the isolates of *E.coli* were sensitive to Imipenem of 96.71%, Nitrofurantoin of 92.41%, Amikacin at 90.89%, Piperacillin-tazobactam at 80.76%, Gentamicin of 59.24%, and Azetreonam of 54.43%. According to the European Urological Association guidelines (28), nitrofurantoin is recommended for the treatment of uncomplicated cystitis as first-line empiric therapy. A study in Iraq (29) revealed that *E. coli* isolates were not resistant to imipenem, ciprofloxacin, amikacin, levofloxacin, and piperacillin, but, this study does not agree with this study, which demonstrated the presence of resistance to these antibiotics. The present study agrees with that of Naziri *et al* (30), which showed the lowest rate of resistance against aminoglycosides (16.7% gentamicin, 21.8% amikacin). As formerly reported, gentamicin and amikacin continued to have activity against the uropathogenic *Escherichia coli* (31). The present study showed that 29% of isolates are multidrug-resistant (MDR) to antibiotics. A study carried out in Romania revealed 35% of the examined strains were multidrug-resistant (32). The multidrug resistance (MDR) phenotype is due to the presence of large plasmids that usually carry resistance genes to β-lactams, Quinolones, aminoglycosides, and co-trimoxazole (32). The prevalence of MDR-UPEC in Iraq is almost average compared to other countries, although, Asian and African countries have been reported to have the highest rates of MDR-UPEC (33). All studies differed in rates of resistance of bacteria causing UTIs and this may be attributed to many factors, like the study population and differences in geographic location. One of the reasons for the development of bacterial resistance to antibiotics is the indiscriminate use of antibiotics by the patient without any medical advice. This leads to the emergence of mutated strains enhancing their ability to prevent drugs from reaching them. Several studies have shown that most urinary tract pathogens have altered their resistance to change over time in many countries (34). In this study, an increase in sensitivity rates to some antibiotics other than the beta-lactam group can be highly reliable in the treatment of *E. coli* that produces ESBLs.

Alipour and Jafari (35) showed that the uniplex PCR of the 45 ESBL-producing *E. coli* strains indicated that the bla $_{TEM}$ was the most abundant gene (89%) , followed by CTX-M (27%) and SHV (20%). These genes are common in *Klebsiella pneumonia* and *E. coli* (36). Investigation of these genes is important not only for their ability to hydrolyze beta-lactam antibiotics but also because the plasmids responsible for the production of ESβLs regularly contain genes coding for resistance to other antibiotic groups such as aminoglycosides and fluoroquinolones. Many several studies show that the distribution of ESBL Genes are diverse. Some studies have shown the predominance of the TEM gene with less SHV and CTX-M (37). This study agrees with another study from India, where TEM was predominant followed by SHV in *E. coli* (38) and also agrees with Jena *et al* (39). A study in China showed The major b-lactamase family detected in the ESBL-producing *E. coli* strains was the CTX-M-9 group (48.4%), followed by the CTX-M-1 group (44.2%) and the CTX-M-2 group was not detected (40). Yahaya *et al.* (41), in Maiduguri reported blaSHV (36.4%) as the predominant gene followed by *bla* $_{TEM}$ (31.4%) and *bla* $_{CTX-M}$ (27.3%). A study in Jordan showed a predominantly of CTX-M group 1 (82.7%) followed by SHV type (30.7%) and CTX-M type 9 (28%) (42). From several different studies, it was observed that the dominant gene for ESBL was diverse. Previous reports stated that the most prevalent type of ESBL genes is SHV, TEM, and CTX-M. During the past decade, TEM and SHV types have been reported to be the most common types of b-lactamase genes, but recently, the CTX-M type has spread widely globally compared to TEM and SHV genotypes (43).The differences in results in the prevalence of these studies of ESBLs may be due to various reasons such as differences in the volume and type of antibiotic consumption and differences in the time at which the isolates were collected (44).

The coexistence of various b-lactamases genes within the same isolates has been verified by many researchers (45). Seyedjavadi *et al* (46) and Manoharan *et al* (47) de-

monstrated the co-existence of different ESBL genes within the same isolate. In accordance with former evidence, the coexistence of ESBL genes (i.e., bla_{SHV}, bla_{CTX-M}, and bla_{TEM}) is because they are frequently situated on the plasmid and can be transferred to other bacteria (48).

The study concludes the high spreading of coexistence of more than one gene of Group A β-lactamase genes among uropathogenic *Escherichia coli* causes them to resist many antibiotics. This makes the treatment regimen unusual or hard to be achieved

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