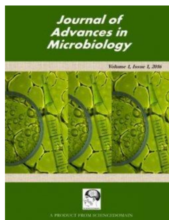

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Antibiotic Resistance Profile of *Escherichia coli* from Urine of Patients with Suspected Urinary Tract Infections in Federal Medical Centre, Keffi, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IHN and YBN designed the study and co-ordinated the research. Authors EBB, GRIP, MDM, RHA, TI and PAT helped with sample collection and laboratory analysis. Author IHN searched for literatures and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the antibiotic resistance profile of *Escherichia coli* from the urine of patients with suspected urinary tract infections in Federal Medical Centre, Keffi, Nigeria.
Study Design: Cross-sectional study.
Place and Duration of Study: Sample was obtained from the Federal Medical Center, Keffi and analyzed at Nasarawa State University, Keffi, Nigeria, between January and April 2018.
Methodology: Three hundred and eighty urine samples were collected and *E. coli* was isolated and identified using standard microbiological methods. Antimicrobial Susceptibility Testing for the

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isolates was carried out and interpreted as described by the Clinical and Laboratory Standards Institute.
Results: The occurrence of the bacterium was 12.9% (49/380). The occurrence in relation to the gender of the patients was higher in the female (15.5%) than the male (9.8%); in relation to age, it was highest at 11-20 years (23.5%) but lowest at > 50 years (2.3%). The isolates were more resistant to ampicillin (81.6%), streptomycin and sulphamethoxazole/ trimethoprime (75.0%) but less resistant to gentamycin (30.6%), and imipenem (22.4%). The occurrences of different classes of resistance were multidrug resistance (MDR) (93.9%) and pan drug resistance (4.2%). Most of the isolates were more resistant to the commonly prescribed antibiotic and were also MDR isolates.
Conclusion: The need to review antibiotic use by the hospital is thus justified.

Keywords: Escherichia coli; urine; antibiotic; resistance.

1. INTRODUCTION

Escherichia coli, a member of the Enterobacteriaceae family, has been reported to be one of the most predominant organisms causing urinary tract infections (UTIs) which are very common reasons for consultation and antibiotic prescription in current practice [1]. Urinary tract infection (UTI) is one of the most frequent types of nosocomial infections and probably affects nearly one-half of all people during their lifetimes [1,2]. Antibiotics such as β-lactams and fluoroquinolones as well as other classes are commonly prescribed for treatment of *E. coli* related UTIs [3,4].

Massive and usually inappropriate use of antibiotics for treatment of UTIs generates a selective pressure that is followed by the rapid emergence and spread of multi-drug resistant bacterial strains [3,4,5]. Nowadays, the resistance of urinary *E. coli* to many antibiotic classes is a very common finding in human medicine and is usually associated with increased medical costs, prolonged hospital stays and frequent therapeutic failure [5].

In addition, several studies showed that antibiotic resistance in *E. coli* related UTIs is increased [2,6]. The emergence of transferable multidrug resistance genes in gram-negative bacteria, particularly *E. coli* is an important health problem throughout the world [2,3,7-10].

Many reports have described and characterized antibiotic-resistant urinary *E. coli* isolates in worldwide [11-15] but in the study location this report is limited, hence this study investigates antibiotic resistance profile of *E. coli* from the urine of Patients with suspected UTIs in Federal Medical Centre, Keffi, Nigeria.

2. MATERIALS AND METHODS

2.1 Isolation of *Escherichia coli*

Escherichia coli was isolated from urine samples as follows: A loopful of urine sample was streaked on MacConkey Agar (Oxoid Ltd. U.K.) plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further streaked on Eosin Methylene Blue Agar (Oxoid Ltd. U.K.) and incubated at 37°C for 24 h. Greenish metallic sheen colonies that grew on the Eosin Methylene Blue agar plate were selected as presumptive *E. coli.*

2.1.1 Identification of *Escherichia coli*

The presumptive *E. coli* was Gram-stained and biochemically identified as suspected *E. coli* using IMViC (Indole, Methyl red, Voges-Proskauer and Citrate) tests as earlier described [16]. The suspected *E. coli* isolates (Gram-negative, rod shape, indole positive, methyl red positive, citrate negative and Voges-Proskauer negative) were using a commercial biochemical testing kit (KB003 H125TM) following the manufacturer's instruction.

2.2 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by the Clinical and Laboratory Standards Institute [17]. Briefly, three (3) pure colonies of the isolates were inoculated into 5 ml sterile 0.85% (w/v) NaCl (BDH chemical Ltd, England) (normal saline) and the turbidity of the bacteria suspension will be adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ (BDH chemical Ltd, England) was added into 99.5 ml of 1% (w/v) H_2SO_4 (BDH chemical Ltd, England).

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar (Oxoid Ltd. U.K.) plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility breakpoint earlier described by Clinical and Laboratory Standards Institute [17].

2.3 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined using the formula: MAR Index = No. antibiotics isolate is resistant to/No. of antibiotics tested as described previously [18].

2.4 Classification of Antibiotic Resistance

Antibiotic resistance in the isolates was classified into: multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [19].

2.5 Statistical Analysis

Microsoft Excel™ 2016 and SPSS version 21 was used as statistical tool for computation of percentages, mean and averages of the data obtained.

3. RESULTS

The cultural, morphological and biochemical characteristics of *E. coli* isolated from urine of the patients is as shown in Table 1.

Out of 380 urine samples obtained from suspected UTI patients, the occurrence of the isolates was 12.9%. The occurrence of the isolate with relation to gender of suspected UTI patients was higher in female (15.5%) than the male (9.8%) as shown in Table 2. There is however no significant difference in the occurrence between males and females since p-value is greater than 0.05. The occurrence of the isolate in relation to age was high in 11-20 years (23.5%) but lower in > 50 years (2.3%) as given in Table 3. The differences in the occurrence of the isolates in relation to gender and age of suspected UTI patients was statistically insignificant (p>0.05).

The isolates were more resistance to ampicillin (81.6%), streptomycin and sulphamethoxazole/ trimethoprime (75.0%) but less resistance to gentamycin (30.6%) and imipenem (22.4%) as shown in Table 4.

The antibiotic resistance phenotypes of *E. coli* isolates from patients with suspected UTI in Federal Medical Centre, Keffi is as shown in Table 5. The isolates were distributed into different antibiotic resistance phenotypes and the most common phenotype was AMC- S-SXT-CTX-CAZ-FOX-CN-CIP-AMP (8.2%).

The MAR index of the isolates from Federal Medical Centre, Keffi is as shown in Table 6. All the isolates were MAR isolates with MAR index of 0.2 and the most common MAR index was 0.8 (20.9%).

The *E. coli* isolates from Federal Medical Centre, Keffi were classified into different categories of antibiotic resistance namely; Multi-drug resistance (MDR), Extensive-drug resistance (XDR) and Pandrug resistance (PDR) as shown in Table 7. The occurrence of different classes of isolate was multidrug resistance (MDR) (93.9%) and pan drug resistance (4.2%).

4. DISCUSSION

The occurrence of *E. coli* from the urine of suspected UTIs patients in the study center was an indication that the organism may be responsible for UTIs and this finding agrees with the study earlier reported by Elboumri et al. [1] and Tajbahsh et al. [1] that *E. coli* is the most frequent uropathogen that causes UTIs nearly one half of all people during their lifetimes.

This occurrence of *E. coli* from the urine of suspected UTIs patients was in higher in female than male patients in both study centers and this finding, however, is not different from the study earlier described by Shakya et al. [14]. The percentage occurrence of *E. coli* from urine of suspected female and male UTIs patients was lower than 78.9% and 21.1% reported by Shakya et al. [14].

The high occurrence of *E. coli* in female than male patients may be due to anatomical differences, hormonal effect, and behavioral patterns [14]. Although, our findings also show the difference in the occurrence of *E. coli* in relation to the gender of suspected UTIs patients in the study center were statistically insignificance and this however shows that gender of an individual may not necessarily be a predisposing factor for UTIs caused by *E. coli.*

Table 1. Cultural, morphological and biochemical characteristics *Escherichia coli* from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria

Cultural characteristics	Morphological characteristics		Biochemical characteristics												Inference
	Gram stain	Morphology	ONPG	Ornithine	UR	LYS	NT	H_2S	CT	TDA	VP	MR	IND	MAL	
Pinkish colony on MCA and greenish metallic sheen colony EMB agar	-	Rod	+	+	-	+	+	-	-	-	-	+	+	_	*E. coli*

MCA = MacConkey agar; EMB = Eosin methylene blue; UR = Urease; LYS = Lysine; H_2S = Hydrogen Sulphide; CT = Citrate; TDA = Phenylalanine deaminase; VP = Voges-Proskauer; IND = Indole; MAL = Malonate; - = Negative; + = Positive

Table 2. Occurrence of *Escherichia coli* from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria in relation to gender

Gender	No. of samples	No. (%) *E. coli*	P-value
Male	173	17(9.8)	0.4596
Female	207	32(15.5)	
Total	380	49(12.9)	

Table 3. Occurrence of *Escherichia coli* from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria in relation to age

Age	No. of samples	No. (%) *E. coli*	P-value
≤10	23	9(13.0)	0.9115
11-20	51	12(23.5)	
21-30	94	8(8.5)	
31-40	106	20(18.7)	
41-50	62	5(8.1)	
>50	44	1(2.3)	
Total	380	49(12.7)	

Table 4. Antibiotic resistance of *Escherichia coli* from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria

Antibiotics	Disc content (µg)	No. (%) resistance (n=49)
Amoxicillin/clavulanic acid (AMC)	30	20(40.8)
Ampicillin (AMP)	30	40(81.6)
Ceftazidime (CAZ)	30	23(46.9)
Cefotaxime (CTX)	30	28(57.1)
Cefoxitin (FOX)	30	26(53.1)
Ciprofloxacin (CIP)	5	28(57.1)
Gentamicin (CN)	10	15(30.6)
Imipenem (IPM)	30	11(22.4)
Streptomycin (S)	30	37(75.5)
Sulphamethoxazole/trimethoprim	25	37(75.5)

Table 5. Antibiotic resistant phenotypes of *Escherichia coli* from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria

Antibiotic resistance phenotypes	Frequency (%) (n=49)
FOX	1(2.0)
SXT-FOX-IPM	1(2.0)
SXT-FOX-AMP	1(2.0)
S-FOX-AMP	1(2.0)
S-SXT-AMP	1(2.0)
S-SXT-FOX-AMP	2(4.1)
S-CAZ-FOX-AMP	1(2.0)
S-SXT-CIP-AMP	1(2.0)
S-SXT-CTX-AMP	1(2.0)
AMC-S-CTX-FOX-AMP	1(2.0)
S-SXT-CTX-CAZ-FOX	1(2.0)
S-SXT-CAZ-FOX-AMP	1(2.0)
SXT-FOX-CN-CIP-AMP	1(2.0)
AMC-S-SXT-CIP-AMP	2(4.1)
S-SXT-CN-CIP-AMP	1(2.0)
S-SXT-FOX-UPM-CIP-AMP	1(2.0)
AMC-S-SXT-CTX-FOX-AMP	1(2.0)
S-SXT-CTX-FOX-CIP-AMP	1(2.0)
S-SXT-CTX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-CAZ-CN-AMP	1(2.0)
S-SXT-CTX-CAZ-CIP-AMP	2(4.1)
S-CTX-CAZ-FOX-CN-IMP-AMP	1(2.0)
S-SXT-CTX-CAZ-FOX-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-AMP	1(2.0)
AMC- S-SXT-CTX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-CAZ-CN-CIP-AMP	2(4.1)
AMC- S-SXT-CTX-CAZ-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-CN-CIP-AMP	4(8.2)
AMC-S-CTX-CAZ-FOX-CN-CIP-AMP	1(2.0)
S-SXT-CTX-FOX-CN-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-CN-CIP-AMP	2(4.1)
AMC-SXT-CTX-CAZ-CN-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-IPM-CIP-AMP	1(2.0)
AMC- S-SXT-CTX-CAZ-FOX-CN-IPM-CIP-AMP	2(4.1)

AMC=Amoxillin/Clavulanic acid; S=Streptomycin; SXT=Suphamethoxazole/Trimethoprim; AMP=Ampicillin; CTX=Cefotaxime; CAZ=Ceftazidime; FOX=Cefoxitin; CN=Gentamicin; IPM=Imipenem; CIP=Ciprofloxacin

Table 6. Multiple antibiotic resistance (MAR) index of *Escherichia coli* isolated from urine of patients with suspected urinary tract infections in federal medical centre, Keffi, Nigeria

No. of antibiotics resistance (a)	No. of antibiotic tested (b)	MAR index (a/b)	Frequency (%)
10	10	1.0	2(4.7)
9	10	0.9	1(2.39
8	10	0.8	9(20.9)
7	10	0.7	7(16.3)
6	10	0.6	7(16.3)
5	10	0.5	7(16.3)
4	10	0.4	5(11.6)
3	10	0.3	4(9.3)
2	10	0.2	0(0)
1	10	0.1	1(2.3)

Table 7. Categories of antibiotic resistance in *Escherichia coli* isolated from urine of patients with suspected urinary tract infection in federal medical centre, Keffi, Nigeria

Categories of antibiotic resistance	Frequency (%) (n=50)
NMDR	1(2.0)
MDR	46(93.9)
XDR	0(0.0)
PDR	2(4.7)

NMDR=None Multi-drug resistance; MDR=Multi-drug resistance; XDR=Extensive drug resistance; PDR=Pandrug resistance

The occurrence of *E. coli* from the urine of suspected UTIs patients in this study was not in agreement with the study earlier described by Shakya et al. [14]. The high occurrence of *E. coli* in 11-20 years of patients in Federal Medical Center Keffi may be due to behavioral pattern of individuals especially their level of hygiene in this age group may be low, although Shakya et al. [14] reported high occurrence of *E. coli* in the group; 21-30 (26.0%) and also shown that the high occurrence may be due to fact that individual at this age group are sexually active and may be more prone to UTIs.

The resistance of the isolates from both study center to ampicillin, streptomycin, sulphamethoxazole/trimethoprim, cefotaxime, ceftazidime, and cefoxitin observed in this study was not surprising and this finding agrees with the study earlier reported by Polse et al. [20], Padilla et al. [21] and Alikhani et al. [22]. The percentage resistance of isolates both in the study center to ampicillin was less than 100% and 90% reported by Polse et al. [20] and Shakya et al. [14]. The resistance of the isolates to cefotaxime and ceftazidime was less than 82.4% reported by Padilla et al. [21]. The resistance of isolates to antibiotic mentioned may be due to antibiotic misuses, ineffective empiric antibiotic therapy, poor dosing regimen of antimicrobial agent, and prolong therapy of infection caused by this organism may also likely being the reason for the resistance of antibiotic mentioned [23].

The low resistance of the isolates from both study centers to antibiotics such as gentamicin, imipenem, amoxicillin/clavulanic and ciprofloxacin was expected and this finding also justifies their use for the treatment of infection caused by Gram-negative bacteria. The percentage resistance of the isolates to gentamicin, and imipenem was less than 10.6% and 13.9% and higher 38.08% resistance to ciprofloxacin as earlier reported by Shakya *et al.* [14].

The occurrence of MAR isolates observed in this study was similar to the study earlier reported by Ngwai et al. [18] and Nkene et al. [12]. The occurrence of the MAR isolates in the study location was an indication that the isolates may be more common in the environment where the antibiotics are likely misused [18].

The occurrence of MDR resistance isolates in the study location was expected and this finding is also not different from the study earlier reported by Thakur et al. [24] and Parajuli et al. [25] that MDR *E. coli* responsible for UTIs that is difficult to be treated using antibiotics. The percentage occurrence of MDR isolates observed in this study was higher than 64.9% reported by Parajuli et al. [25]. The occurrence of XDR and PDR resisting isolated observed in this study was also similar to the study earlier described by Parajuli et al. [25]. Most of the isolate was more resistance to commonly prescribed antibiotic and

were also MDR isolates. Further studies on molecular characterization of β-lactam fluoroqunolones resistance in the isolates are ongoing.

5. CONCLUSION

This study recovered 12.9% (49/380) *E. coli* from the urine of patients. The occurrence in relation to the gender of the patients was higher in the female (15.5%) than the male (9.8%); in relation to age, it was highest at 11-20 years (23.5%) but lowest at > 50 years (2.3%). The isolates were more resistant to ampicillin (81.6%), streptomycin and sulphamethoxazole/trimethoprime (75.0%) but less resistant to gentamycin (30.6%), and imipenem (22.4%). The occurrences of different classes of resistance were multidrug resistance (MDR) (93.9%) and pan drug resistance (4.2%). Most of the isolates were more resistant to the commonly prescribed antibiotic and were also MDR isolates. Further investigation on the molecular basis this resistance is ongoing.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. El-Bouamri MC, Arsalane L, Zerouali K, Katfy K, El kamouni Y, Zouhair S. Molecular characterization of extended spectrum lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. African Journal of Urology. 2015;21:161–166.
2. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: A multifaceted threat. Clinical Microbiology Reviews. 2009;22(4):664-89.
3. Jeong HS, Bae IK, Shin JH, Jung HJ, Kim SH, Lee JY. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in Enterobacteriaceae. The Korean Journal of Laboratory Medicine. 2011;31(4):257-64.
4. Ruiz E, Ocampo-Sosa AA, Alcoba-Flórez J. Changes in ciprofloxacin resistance levels in *Enterobacter aerogenes* isolates associated with variable expression of the *aac(6')-Ib-cr* gene. Antimicrobial Agents and Chemotherapy. 2012;56(2):1097–1100.
5. Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L. The ARESC study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. International Journal of Antimicrobial Agents. 2009;34:407–13.
6. Ferjani S, Saidani M, Amine FS, Boubaker IBB. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum *β*-lactamase-producing *Enterobacteriaceae* in a Tunisian hospital. Microbial Drug Resistance. 2015;21(2):158–166.
7. Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, Shahcheraghi F. Distribution of bla TEM, bla SHV, bla CTX-M genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. Microbial Drug Resistance. 2010;16(1):49-53.
8. Bouchakour M, Zerouali K, Claude JDPG. Plasmid-mediated quinolone resistance in expanded-spectrum beta lactamase producing enterobacteriaceae in Morocco. The Journal of Infection in Developing Countries. 2010;4(12):799–803.
9. Crémet L, Caroff N, Dauvergne S, Reynaud A, Lepelletier D, Corvec S. Prevalence of plasmid-mediated quinolone resistance determinants in ESBL Enterobacteriaceae clinical isolates over a 1-year period in a French hospital. Pathologie Biologie. 2011;59(3):151–156.
10. Firoozeh F, Zibaei M, Soleimani-Asl Y. Detection of plasmid-mediated qnr genes among the quinolone-resistant *Escherichia coli* isolates in Iran. The Journal of Infection in Developing Countries. 2014;8(7):818–822.
11. Fortini D, Fashae K, Garcia-Fernandez A, Villa L, Carattoli A. Plasmid-mediated quinolone resistance and β-lactamases in *Escherichia coli* from healthy animals from

Nigeria. Journal of Antimicrobial Chemotherapy. 2011;66:1269-1272.

12. Nkene IH, Ngawi YB, Omede MU, Samuel J, Envuladu EY, Abimiku RH. Extended spectrum beta-lactamase producing *Escherichia coli* from urine of symptomatic and asymptomatic subjects in Keffi, Nigeria. International Journal of Research Studies in Biosciences. 2015;3(12):1-5.
13. Tajbakhsh E, Parvin A, Elham A, Nazila A, Faham K. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrobial Resistance and Infection Control. 2016;5(11):2-8.
14. Shakya P, Shrestha D, Maharja E, Sharma VK, Paudyal R. Extended-spectrum β-lactamase producing among *Escherichia coli* and *Klebsiella* spp causing urinary tract infections; A hospital-based study. Open Microbiology Journal. 2017;11:23-30.
15. Eghieye MO, Jodi SM, Bassey BE, Nkene IH, Abimiku RH, Ngwai YB. Antimicrobial resistance profile of *Escherichia coli* isolated from urine of patients in selected General Hospitals in Abuja Municipal, Nigeria. Asian Journal of Advanced Research and Report. 2018;2(2):1-10.
16. Cheesebrough M. Medical laboratory manual for tropical countries. Cambridge: Cambridge University Press. 2006;49-97.
17. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 27th Informational Supplement M100-S22. Wayne, Pa, USA; 2017.
18. Ngwai YB, Gyar SD, Pennap GRI, Makut MD, Ishaleku D, Corosi SM, Nkene IH, Uzoamaka N. Antibiogram of non-sorbitol fermenting *Escherichia coli* isolated from environmental sources in Keffi, Nigeria. NSUK Journal of Science and Technology. 2014;4(1&2):152-163.
19. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, Olsson-Liljequist B. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012;18(3): 268-281.
20. Polse RF, Yousif SY, Assafi MS. Prevalence and molecular characterization of extended-spectrum β-lactamase producing uropathogenic *Escherichia coli* isolated in Zakho, Iran. Journal of Microbiology and Infectious Diseases. 2016;6(4):163-167.
21. Padilla E, Llobet E, Doménech-Sánchez A, Martínez-Martínez L, Bengoechea JA, Albertí S. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrobial Agents and Chemotherapy. 2010;54(1): 177–183.
22. Alikhani MY, Tabar ZK, Mihani F, Kalantar E, Karami P, Sadeghi M, Farajnia S. Antimicrobial resistance patterns and prevalence of blaPER-1 and blaVEB-1 genes among ESBL-producing *Pseudomonas aeruginosa* isolates in West of Iran. Jundishapur Journal of Microbiology. 2014;7(1).
23. Assafi MS, Ibrahim NMR, Hussein NR, Taha AA, Balatay AA. Urinary bacterial profile and antibiotic susceptibility pattern among patients with urinary tract infections in Duhok City, Kurdistan Region, Iraq. International Journal of Pure and Applied Science and Urology. 2015;30:54-63.
24. Thakur S, Pokhoel N, Sharma M. Prevalence of multi-drug resistant enterobactericeae and extended-spectrum β-lactamase producing *Escherichia coli* in urinary tract. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(4):1615-1624.
25. Parajuli NP, Maharjan P, Parajul H. High rate of multi-drug resistance among uropathogenic *Escherichia coli* in children and analysis of extended-spectrum β-lactamase producers from Nepal. Antimicrobial Resistance, Infection and Control. 2017;6(1):9.

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