JOURNAL OF TECHNIQUES

Journal homepage: http://journal.mtu.edu.iq

RESEARCH ARTICLE - MEDICAL TECHNIQUES

Antibiogram Pattern of Uropathogenic *Escherichia Coli* in Baghdad Province, Iraq

Nuha Abd Al.rheem Saad[1,2*], Raghad Hassan Hussein[1], Nadia M. M. Al-Shakir[1]

[1] College of Health & Medical Technology - Baghdad, Middle Technical University, Baghdad, Iraq

[2] Oncology teaching hospital- Baghdad, Ministry of Health and Environment, Iraq

[*] Corresponding author E-mail: nuhabaghdad2@gmail.com

Article Info.

Article history:

Received
13 July 2022

Accepted
30 July 2022

Publishing
15 November 2022

Abstract

Uropathogenic *Escherichia coli* (UPEC) is considered one of the main causes of urinary tract infections. Antimicrobial resistance (AMR) is a significant global health care issue, particularly with regard to urinary tract infections. Objective: To evaluate the prevalence of antibiotic resistance among *Escherichia coli* isolated from patients with urinary tract infection.

Two-hundred and sixty-four mid-stream urine samples were collected from patients with symptoms of urinary tract infection who visited Baghdad teaching Hospital. These samples were routinely cultured on different media and *E. coli* was identified using conventional methods and confirmed by VITEK-2 system. Following diagnosis, 10 different types of antibiotics were tested for their sensitivity on *E. coli* strains using the Kirby-Bauer disk diffusion method. Results: Out of 264 urine samples, 175 (66%) contained Gram-negative bacteria, *E. coli* was the most common uropathogenic isolate (38%), followed by K. pneumoniae (16%) and Streptococcus epidermidis (12%). The majority of uropathogenic *E. coli* showed the most rate of resistance to Amoxicillin, ceftriaxone, cefotaxime, Nalidixic acid, Trimethoprim/Slfamethoxazole & tetracycline (88%, 80%,75%, 67%, 65.0% and 57% respectively). Ninty-three percentage were sensitive to Meropenem, followed by Nitrufurnantion and Chloramphenicole (75.0% and 68.0%) respectively. Conclusions: It was concluded from this study that *E. coli* is the main pathogen inflicting UTIs on patients. Amoxicillin, ceftriaxone, cefotaxime, Nalidixic acid, Trimethoprim/Slfamethoxazole & tetracycline were among the antibiotics with the highest rates of resistance. In light of this study, local sensitivity patterns rather than international guidelines should be the basis for empirical antibiotic therapy.

Publisher : Middle Technical University

Keywords: UPEC; Antibiotics resistance; UTI; Amoxicillin; Meropenem.

1. Introduction

Infection of the urinary tract is among the most prevalent diseases, frequently caused by facultative gram-negative bacteria, which are characterized by bacterial colonization of the urinary system [1]. UTIs can occur in both genders at any age during their lifetime. However, it occurs more frequently in females than in males. The most frequent risk factors for UTIs are frequent intercourse, multiparty relationships, advanced age, abnormalities of the urinary tract, and low socioeconomic levelin addition to past UTI treatment [2, 3]. In addition to UPEC, UTIs can also be caused by *Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, and Citrobacter* species. These pathogens were considered to be classic uropathogens [4]. It also is probable for susceptible bacteria to develop resistance to an antimicrobial agent by mutation, degradation, or alteration of the antibiotic's structure, the efflux pump, conjugation, and transformation of the plasmid [5].

The antimicrobial-resistant strains of *E. coli* have become a worldwide dangerous health issue, because it decreases the activity of the antibiotic agent, hence increases the severity of infection, and rises the rate of mortality and morbidity [6]. Possible reasons are that UTI is usually treated without urine culture or antibiotic sensitivity testing to select the correct antimicrobial agents. The prolonged use of empirical treatment lead to complicated outcomes in patients with chronic recurrent UTIs, including rising of antibiotic resistance to the causative agents [7]. The World Health Organization (WHO) has stated that antibiotic usage or abuse is the cause of the high degree of AMR seen in the human population. Approximately 50–60% of community-acquired *E. coli* infections have developed resistance to widely used oral antibiotics. (e.g. Amoxicillin, Cefixime, and Ciprofloxacin), making outpatient treatment difficult [8]. Due to its significant health impact, local antibiotic sensitivity data regarding community acquired UTIs is regularly needed to ensure efficient treatment of such infection. Thus, the current study was aimed at providing such data for frequent pathogens.

Nomenclature			
AMR	Antimicrobial resistance	WHO	World Health Organization
CLSI	Clinical and Laboratory Standards Institute	CHL	Chloramphenicole
STX	Trimethoprim/Slfamethoxazole	AMC	Amoxicillin
MEM	Meropenem	EMB	Eosin methylen blue
UTI	Urinary tract infection	CTX	Cefotaxime
UPEC	Uropathogenic *Escherichia coli*	CRO	Ceftriaxone
NFN	Nitrufurnantion	TE	Tetracycline
CN	Gentamicim		

2. Material and Methods

2.1. Specimen collection

This study was conducted during the period from November/2021 to January/2022. A total of 264 midstream urine samples were taken from patients (223 females and 41 males) with an age range between 5 to 67 years, who had signs and were primarily diagnosed with UTI in Baghdad Teaching Hospital. Ethical approval to conduct this study was obtained from the Iraqi of Ministry of Health (no A9226).

2.2. Isolation of E. coli by traditional methods

In the laboratory and under aseptic condition, the collected urine sample were streaked directly on MacConky agar, blood agar and Eosin methylene blue (EMB) agar after preparation of this culture media according to guidelines of the manufacture and sterilization by autoclave. Samples were then incubated for 24h at 37°C to identify isolated colonies of *E. coli* based on their the morphological characteristics, gram staining and some biochemical properties.

2.3. Identification of E. coli with VITEK- 2 system

To verify/confirm the diagnosis, the bacterial isolates were inoculated, cultured, and then incubated at 37°C for an overnight period. The turbidity of the bacteria suspension was adjusted with VITEK densities (bio-Merieux) to match the McFarland (0.5-0.7) standard after which a single colony was taken and suspended in the solution. The bacterial suspension tubes were then manually loaded into the VITEK-2 system, and the remaining software steps were carried out as via the manufacturer's instructions (bio-Merieux).

2.4. Antibiotic susceptibility test

The antimicrobial resistance to ten antibiotics (amoxicillin 10 mg, chloramphenicol 30 mg, ceftriaxone 30 mg, cefotaxime 30 mg, gentamicin 10 mg, STX 1.25 mg/23.75 mg, Meropenem 10 mg, Nalidixic acid 30 mg, Nitrofurantoin 300 mg and tetracycline 30 mg) were evaluated using Kirby Bauer's disc diffusion method on Muller-Hilton a (Hi-Media laboratories). The clear zone widths for these disks against Enterobacteriaceae in the guidelines of the Clinical and Laboratory Standards Institute (CLSI) were compared to the antibiotic disks' zone of inhibition [9].

2.5. Statistical analyses

The SPSS statistical software (version 26) was used for statistical analyses, and the Chi-square test was used to assess qualitative relationships. Statistical significance was defined as a *P*-value less than 0.05.

3. Results

From 264 urine samples taken from patients, significant bacteriuria was observed in 175 (66%), while 17 (7%) cultures gave fungal growth. On the other hand, 29 (11%) specimens showed mixed/ contaminated growth. Furthermore, 43 (16%) of the samples showed no growth, as shown in Fig. 1.

The prevalence of bacterial growth revealed that *E. coli* was the most common bacterial isolated pathogen 100 (38%) as shown in Table 1. The next prevalent pathogens were K. pneumoniae at 43(16%) and S. epidermidis 23(9%). Additionally, urinary tract candidiasis (C. albicans) was the most prevalent nosocomial fungal infection at 17 (7 %). Whereas, S. aureus was only found in 9(3%) samples.

Distribution of UPEC according to gender and age showed that the prevalence of bacterial UTI was highest in the age group 20–34 years (33%) compared to the oldest age group of 65–79 years, which showed the lowest frequency of only 10% (Table 2). Urinary tract infection was highest in females (75%) as compared to (25%) males.

Distribution of *E. coli* isolates according to antibiotic resistance (antibiogram pattern) is presented in Fig. 2 Which showed that *E coli* has the most resistance rate to amoxicillin 88%, followed by Ceftriaxon at 80% and 75%, 67%, 65% and 57% for CTX, Nalidixic acid, STX and Tetracycline respectively. Nevertheless, UPEC recorded low resistance against Gentamicin (24.0%), Chloramphenicole (19.0%), Nitrufurnantion (7.0%) and Meropenem (6.0%). Whereas, the most of *E. coli* isolates were susceptible to Meropenem, Nitrufurnantion and Chloramphenicole (93.0%, 75.0% and 68.0%) respectively.

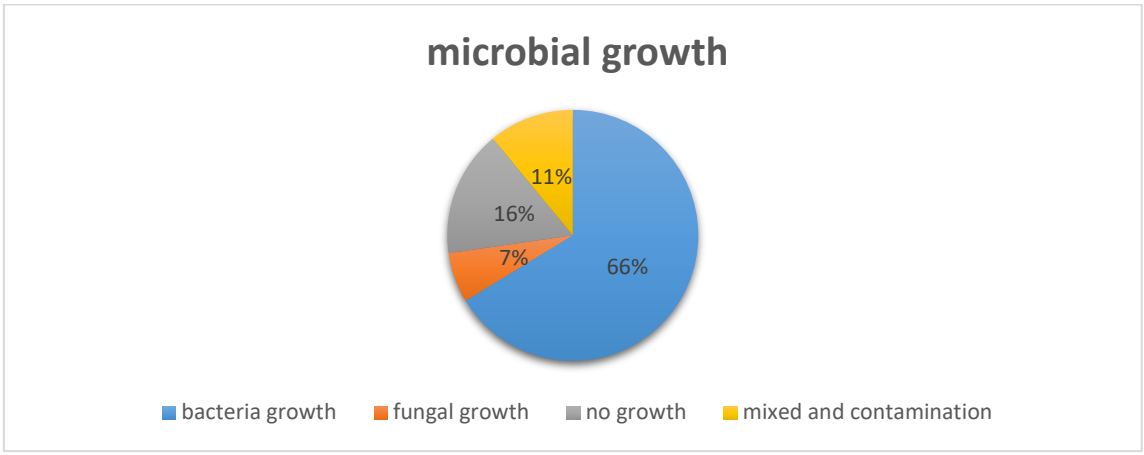

Fig 1. Cultural results of the samples included in the study

Table 1 Prevalence of microbial isolates in the study

Causative agents	No. of Samples	Percentage (%)
E.coli	100	38
K. . pneumoniae	43	16
S. epidermidis	23	9
C. albicans	17	6
S. aureus	9	3
Mixed and Contaminated Growth	29	11
No Growth	43	16
Total	264	100

Table 2 the prevalence of *E. coli* isolates according to age and gender

Age group /Years	Gender		P-value
	Male (N=25)	Female (N=75)	
(5-19)	12 (12%)		
(20-34)	33 (33%)		
(35-49)	30 (30%)		0.002**
(50-64)	15 (15%)		
(65-79)	10 (10%)		
Age (Mean±SD)	38.49±17.20		

**=highly significant

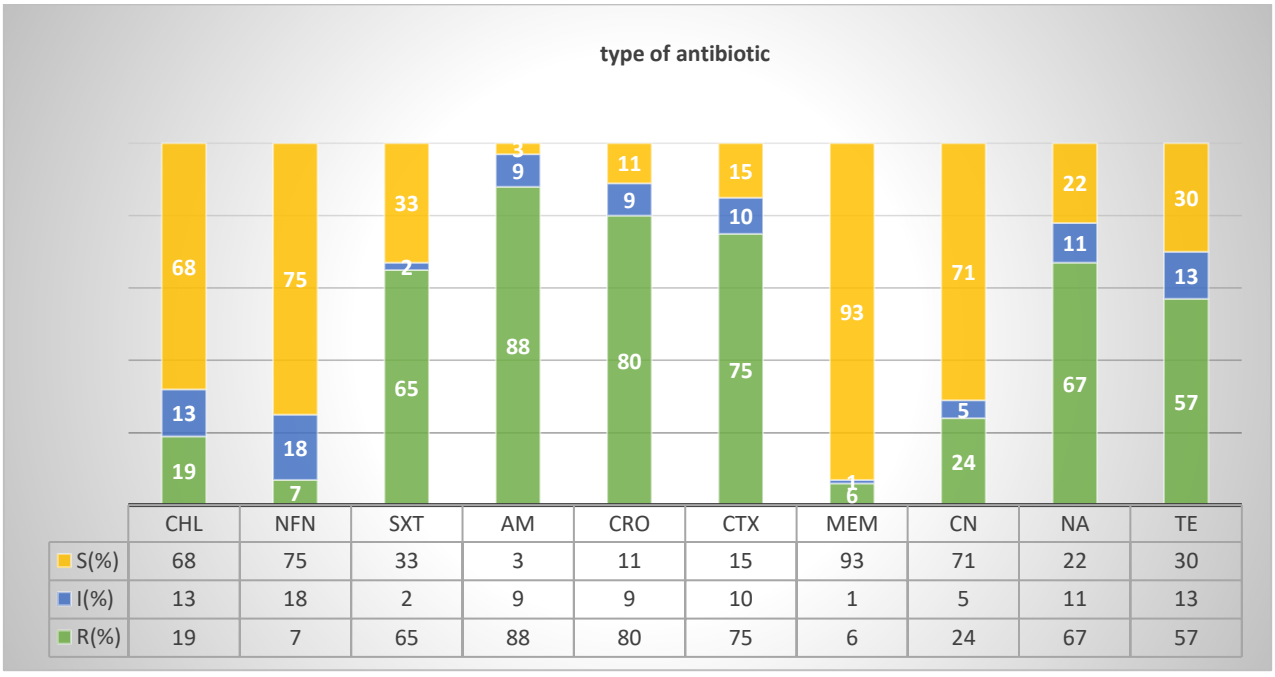

Fig 2. Antibiogram susceptibility profiles of *E. coli* isolates (N=100)

4. Discussion

Antibiotic resistance especially in pathogens causing urinary tract infection is increasing at an escalating level in most parts of the world which highlights the importance of timely diagnosis and efficient treatment of such infections. The results of the present study corresponded to the result reported in Duhok Iraq [10] confirming that the majority (80.6%), of the microorganisms were Gram-negative. Furthermore, the results also corresponded with [11, 12] who have ensured that UTI are common infections caused by bacteriuria. Previous studies in Iraq [13] and [14] also found that *E. coli* is the most common pathogen, which is associated with both community- and hospital-acquired UTI in many countries. Several reasons make *E. coli* the most prevalent pathogen such as being among the normal microbiota of the human intestines, but when these bacteria enter the urethra, it will cause a UTI [15].

However, the result of the current study disagrees with [16] that found *K. pneumonia* as the most important cause of UTI. In the present study, *S. epidermidis* was ranked as the third most causative agent of UTI with a frequency and percentage of 23 (9%), which normally occurs on the skin of humans, as a part of the human normal microbiota [17]. *Staphylococcus epidermidis* was considered an opportunistic pathogen and causes UTIs mostly in young women and the elderly, but rarely in children [18]. The development of antifungal-resistant Candida species is a result of the rising prevalence of UTIs caused by Candida species [19].

The high female percentage compared to males may be attributed to anatomical reasons, females are more prone to developing urinary tract infections. Factors that increase the risk of urinary tract infections in females include physiological factors related to the nature of the female genital tract (the proximity of the vagina to the perianal region and the urethra's shortness). While the longer length of urethra in males and anti-bacterial activity of the prostatic fluid make males less prone to UTI [20]. The present result is consistent with the results of a recent study which reported a higher incidence of UTI caused by *E. coli* in females [21]. Although infection of the urinary tract can effect individuals of all age groups, but age associated trends shows that it is most prevalent in the age group 20-40 years which is consistent with the present findings [22]. UTI is usually treated without urine culture or antibiotic sensitivity testing to select the correct antimicrobial agents which has led to rising of antibiotic resistance to the causative agents[23].

The outcome of antibiotic resistance of 100 uropathogenic *E. coli* isolates examined for their susceptibility to ten antibiotics showed results that corroborated with the findings of previous reports [24] which found that the organism had a high resistance to first-line antibiotics (Gentamicin and nitrofurantoin). Furthermore, it agreed with a recent study on *E. coli* [25] which considered that Meropenem and Imipenem should be the primary medications for *E. coli* infections identified from clinical samples. Furthermore, it agreed with [26] which observed that ampicillin and AMC has the most common antibiotic resistance rate. Moreover, studies conducted by [3, 27] showed high resistance rates of *E. coli* to STX indicating that due to the high prevalence of resistant UPEC to this antimicrobial agent highlighting that this antibiotic should no longer be used for UTI therapy. Whilst [28] showed that resistance to AMC, tetracycline, nalidixic acid was high. Most antibiotic resistance rates may be due to uncontrolled consumption as a result of easy access to ineffective and cheap antibiotics. Furthermore, insufficient adherence to infection control guidelines, as well as inappropriate antibiotic use, could justify this [29].

4. Conclusion

In the present study, *E. coli* was the most common Gram-negative uropathogen causing UTIs followed by K. pneumoniae, and Streptococcus spp. Antimicrobial susceptibility profiles proved that Meropenem and nitrofurantoin are the most appropriate antibiotics for empirical therapy for uropathogenic *E. coli*. Whereas, AMC and CRO antibiotics should be the last choices for treatment which make the treatment very difficult. Regular and continuous monitoring to choose the best antibiotics for empirical treatment, sensitivity patterns must be carried out.

Acknowledgment

All staff in the Laboratories of Baghdad Medical City hospital are acknowledged for their help.

Reference

[1] P. Behzadi, E. Urbán, and M. Gajdács, "Association between biofilm-production and antibiotic resistance in uropathogenic Escherichia coli (UPEC): an in vitro study," Diseases, vol. 8, p. 17, 2020.

[2] S. S. Fatima and E. A. Mussaed, "Urinary tract infection," in Bacterial Identification and Drug Susceptibility Patterns in Pregnant and Non Pregnant UTI Patients, ed: Springer, pp. 1-22, 2018.

[3] G. Shaheen, M. Akram, F. Jabeen, S. M. Ali Shah, N. Munir, M. Daniyal, et al., "Therapeutic potential of medicinal plants for the management of urinary tract infection: A systematic review," Clinical and Experimental Pharmacology and Physiology, vol. 46, pp. 613-624, 2019.

[4] L. A. Ismael, S. H. Aubaid, and H. M. Nasir, "Estimating the Level of Interleukin-22 in Sera of Patients with Uropathogenic Escherichia coli Infection in Mosul City," Rafidain journal of science, vol. 31, 2022.

[5] S. Raimondi, L. Righini, F. Candeliere, E. Musmeci, F. Bonvicini, G. Gentilomi, et al., "Antibiotic Resistance, Virulence Factors, Phenotyping, and Genotyping of E. coli Isolated from the Feces of Healthy Subjects," Microorganisms, vol. 7, Aug 10 2019.

[6] R. Thänert, K. A. Reske, T. Hink, M. A. Wallace, B. Wang, D. J. Schwartz, et al., "Comparative genomics of antibiotic-resistant uropathogens implicates three routes for recurrence of urinary tract infections," MBio, vol. 10, pp. e01977-19, 2019.

[7] H. J. Ho, M. X. Tan, M. I. Chen, T. Y. Tan, S. H. Koo, A. Y. Koong, et al., "Interaction between antibiotic resistance, resistance genes, and treatment response for urinary tract infections in primary care," Journal of clinical microbiology, vol. 57, pp. e00143-19, 2019.

[8] D. S. Lee, S.-J. Lee, and H.-S. Choe, "Community-acquired urinary tract infection by Escherichia coli in the era of antibiotic resistance," BioMed research international, vol. 2018, 2018.

[9] I. Gajic, J. Kabic, D. Kekic, M. Jovicevic, M. Milenkovic, D. Mitic Culafic, et al., "Antimicrobial susceptibility testing: A comprehensive review of currently used methods," Antibiotics, vol. 11, p. 427, 2022.

[10] I. A. Naqid, A. A. Balatay, N. R. Hussein, H. A. Ahmed, K. A. Saeed, and S. A. Abdi, "Bacterial strains and antimicrobial susceptibility patterns in male urinary tract infections in Duhok province, Iraq," Middle East Journal of Rehabilitation and Health Studies, vol. 7, 2020.

[11] F. M. Al-Asady, D. A. Al-Saray, and A. E. Al-Araji, "Screening of urinary tract bacterial infections and their antibiogram among non-pregnant women admitted to Al-Sadiq hospital, Iraq," in AIP Conference Proceedings, p. 020006, 2022.
[12] N. A. J. Al-Tulaibawi, "Prevalence and sensitivity of bacterial urinary tract infection among adult diabetic patients in misan province, Iraq," J Pure Appl Microbiol, vol. 13, pp. 847-853, 2019.
[13] M. T. Hasan, K. K. Ghaima, A. A. Abdulhassan, and M. K. Alwan, "Prevalence and Antimicrobial Susceptibility of Gram-negative Bacteria in Pediatric Patients with Urinary Tract Infections in Baghdad Hospitals, Iraq," Annals of the Romanian Society for Cell Biology, vol. 25, pp. 6102-6109, 2021.
[14] I. Odongo, R. Ssemambo, and J. M. Kungu, "Prevalence of Escherichia Coli and its antimicrobial susceptibility profiles among patients with UTI at Mulago Hospital, Kampala, Uganda," Interdisciplinary Perspectives on Infectious Diseases, vol. 2020, 2020.
[15] V. M. Sora, G. Meroni, P. A. Martino, A. Soggiu, L. Bonizzi, and A. Zecconi, "Extraintestinal pathogenic escherichia coli: virulence factors and antibiotic resistance," Pathogens, vol. 10, p. 1355, 2021.
[16] R. F. Polse, S. M. Qarani, M. S. Assafi, N. Sabaly, and F. Ali, "Incidence and Antibiotic Sensitivity of Klebsiella pneumonia isolated from urinary tract infection patients in Zakho emergency hospital/Iraq," Journal of Education and Science, vol. 29, pp. 257-268, 2020.
[17] M. Otto, "Molecular basis of Staphylococcus epidermidis infections," in Seminars in immunopathology, pp. 201-214, 2012.
[18] O. Megged, "Staphylococcus aureus urinary tract infections in children are associated with urinary tract abnormalities and vesico-ureteral reflux," Pediatric Nephrology, vol. 29, pp. 269-272, 2014.
[19] M. Gharaghani, S. Taghipour, M. Halvaeezadeh, and A. Z. Mahmoudabadi, "Candiduria; a review article with specific data from Iran," Turkish journal of urology, vol. 44, p. 445, 2018.
[20] M. J. Bono and W. C. Reygaert, "Urinary tract infection," in StatPearls [Internet], ed: StatPearls Publishing, 2021.
[21] M. Ibrahim, H. M. Khalid, and W. M. Mer, "The Prevalence of Uropathogenic Escherichia coli Strains among Outpatients with Urinary Tract Infection in Zakho Hospitals-Zakho City, Duhok Province/Iraq," Al-Qadisiyah Journal of Pure Science, vol. 26, pp. 26-40, 2021.
[22] S. Cardone, C. Petruzziello, A. Migneco, B. Fiori, T. Spanu, T. D'Inzeo, et al., "Age-related trends in adults with urinary tract infections presenting to the emergency department: a 5-year experience," Reviews on recent clinical trials, vol. 14, pp. 147-156, 2019.
[23] A. Klingeberg, I. Noll, N. Willrich, M. Feig, D. Emrich, E. Zill, et al., "Antibiotic-Resistant E. coli in Uncomplicated Community-Acquired Urinary Tract Infection: A Prospective Cohort Study from 2015/16 (the SARHA Study) Compared With Data From the Antimicrobial Resistance Surveillance System (ARS)," Deutsches Ärzteblatt International, vol. 115, p. 494, 2018.
[24] T. Zwane, L. Shuping, and O. Perovic, "Etiology and antimicrobial susceptibility of pathogens associated with urinary tract infections among women attending antenatal care in four South African tertiary-level facilities, 2015–2019," Antibiotics, vol. 10, p. 669, 2021.
[25] A. R. Mulakhudair, K. A. Hadi, and A. R. Mulakhudair, "Isolation, identification and detection of some acriflavine (Acr) Efflux pumps genes in uropathogenic E. coli isolates isolated from Iraqis patients," Annals of Tropical Medicine and Health, vol. 23, pp. 232-119, 2020.
[26] N. Daoud, M. Hamdoun, H. Hannachi, C. Gharsallah, W. Mallekh, and O. Bahri, "Antimicrobial susceptibility patterns of Escherichia coli among Tunisian outpatients with community-acquired urinary tract infection (2012-2018)," Current urology, vol. 14, pp. 200-205, 2020.
[27] M. A. K. Shakhatreh, S. F. Swedan, M. e. A. Al-Odat, and O. F. Khabour, "Uropathogenic Escherichia coli (UPEC) in Jordan: Prevalence of urovirulence genes and antibiotic resistance," Journal of King Saud University - Science, vol. 31, pp. 648-652, 2019/10/01/ 2019.
[28] J.-P. Lavigne, F. Bruyère, L. Bernard, C. Combescure, E. Ronco, P. Lanotte, et al., "Resistance and virulence potential of uropathogenic Escherichia coli strains isolated from patients hospitalized in urology departments: a French prospective multicentre study," Journal of Medical Microbiology, vol. 65, pp. 530-537, 2016.
[29] J. A. Ayukekbong, M. Ntemgwa, and A. N. Atabe, "The threat of antimicrobial resistance in developing countries: causes and control strategies," Antimicrobial Resistance & Infection Control, vol. 6, pp. 1-8, 2017.