https://doi.org/10.31925/farmacia.2023.1.19 **ORIGINAL ARTICLE**

# URINARY TRACT INFECTIONS IN A ROMANIAN POPULATION: ANTIMICROBIAL RESISTANCE OF UROPATHOGENS – A MULTIREGIONAL STUDY

AUREL RUSU [1#], AIDA PETCA [2#], CRISTIAN MAREȘ [3#], RĂZVAN-COSMIN PETCA [3*], RĂZVAN-IONUȚ POPESCU [3], SILVIUS NEGOIȚĂ [4], RĂZVAN-ALEXANDRU DĂNĂU [3], CĂLIN BOGDAN CHIBELEAN [5], VIOREL JINGA [3]

[1]*Department of Urology, Vaslui Emergency County Hospital, Vaslui, Romania*
[2]*Department of Obstetrics and Gynecology, Elias Emergency Hospital,"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[3]*Department of Urology," Prof. Dr. Th. Burghele" Clinical Hospital,"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[4]*Department of Anesthesiology and Critical Care, Elias University Hospital,"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
[5]*Department of Urology, Mureș County Hospital, George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu-Mures, Târgu-Mureș, Romania*

**corresponding author: razvan.petca@umfcd.ro*
*[#]Authors with equal contribution.*

*Manuscript received: November 2022*

## Abstract

Urinary Tract Infections (UTIs) are a serious public health problem determined by a large variety of microorganisms; the leading uropathogen is represented by *Escherichia coli*, followed by *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp. as major Gram-negative bacteria. *Enterococcus* spp. and *Staphylococcus* spp. are the most frequent Gram-positive pathogens involved. The presented study aimed to corroborate results from four tertiary hospitals in three different regions of Romania. Analysing 15,907 urine probes, of which 2,842 (17.86%) met the inclusion criteria, for discovering the uropathogen involved in the dynamics of UTIs, desiring to determine the resistance and sensitivity patterns for each pathogen presented, in a comparative approach. *Escherichia coli* was the leading pathogen 1629 (57.31%), presenting the highest resistance to levofloxacin R = 20.62% and amoxicillin - clavulanic acid R = 18.17%; the second most frequent bacteria were *Klebsiella* spp. 530 (18.64%), with major resistance to amoxicillin-clavulanic ac. R = 45.66% and ceftazidime R = 29.05%. *Enterococcus* spp. was the most common Gram-positive strain 340 (11.96%), presenting important resistance to levofloxacin R = 32.35% and penicillin R = 25.29%. Current guidelines recommend adjusting treatment to local data in the management of UTIs; thus, this study involving all three major regions of Romania represents a pivotal point in providing an appropriate recommendation for every health professional.

## Rezumat

Infecțiile tractului urinar (ITU) reprezintă o problemă gravă de sănătate publică determinată de o mare varietate de microorganisme; uropatogenul principal este reprezentat de *Escherichia coli*, urmat de *Klebsiella* spp., *Proteus* spp. și *Pseudomonas* spp. ca bacterii Gram-negative. *Enterococcus* spp. și *Staphylococcus* spp. sunt agenții patogeni Gram-pozitivi implicați cel mai frecvent. Studiul prezentat și-a propus să coroboreze rezultatele a patru spitale terțiare din trei regiuni diferite ale României, analizând 15.907 probe urinare dintre care 2.842 (17,86%) au îndeplinit criteriile de includere, pentru determinarea uropatogenului implicat în dinamica ITU și determinarea rezistenței și sensibilității fiecărui agent patogen prezentat, într-o abordare comparativă. *Escherichia coli* a fost principalul agent patogen 1.629 (57,31%), prezentând cea mai mare rezistență la levofloxacină R = 20,62% și amoxicilină-clavulanic ac. R = 18,17%; a doua cea mai frecventă bacterie a fost *Klebsiella* spp. 530 (18,64%), cu rezistență majoră la amoxicilină - acid clavulanic. R = 45,66% și ceftazidimă R = 29,05%. *Enterococcus* spp. a fost cea mai frecventă tulpină Gram-pozitivă 340 (11,96%), prezentând rezistență importantă la levofloxacină R = 32,35% și penicilină R = 25,29%. Ghidurile actuale recomandă ajustarea tratamentului la datele locale în managementul ITU; astfel, acest studiu care implică toate cele trei regiuni majore ale României reprezintă un punct cheie în furnizarea de recomandări adecvate pentru fiecare profesionist din domeniul sănătății.

**Keywords**: urinary tract infection, AMR, antibiotic, resistance, uropathogens, *Escherichia coli*, *Klebsiella*, *Enterococcus*

## Introduction

Urinary tract infections (UTIs) are a common bacteriological finding in both male and female populations worldwide, causing morbidity in more than 150 million patients yearly [14, 24]; it represents one of the most common outpatient infections. Half of the adult women will experience an episode of UTIs during their lifetime, and around 10% of them in the postmenopausal period had an acute infection during last year [3]. The prevalence of UTIs increases with age, except for a spike in 14 - 24 years old young women; the latter are reported to present up to 30% chances of developing an episode of UTIs [12]. The elderly and urinary bladder catheterization requiring patients are also susceptible [53].

The most frequent form of infection is uncomplicated cystitis in sexually active women with early recurrences. After the first episode of disease, 27% will experience a recurrence in less than half of one year, and almost 3% will develop a new episode during the first year [33]. There are estimated nearly a quarter of a million cases of acute pyelonephritis in young women, only in the United States alone, yearly; almost 10% developing a more severe form requiring hospital admission [33]. The recurrence rates are lower than uncomplicate d UTIs, considering less than 10% of the studied population, both males and females, having a second episode during the next 12 months [3].

The European Association of Urology (EAU) suggests the ORENUC classification system of UTIs based on the anatomical site of infection, the clinical presentation, the risk factors categorization, the severity of the disease, and the appropriate antibiotic therapy [4]. Clinically, UTIs are defined as uncomplicated and complicated UTIs (cUTIs). In an uncomplicated UTI, it is generally accepted that the infection is limited to the bladder, causing cystitis in young and otherwise healthy females with no known risk factors and abnormalities of the genitourinary tract. cUTIs are considered to occur in all men, usually involving the upper urinary tract causing pyelonephritis. They may complicate evolution in each gender. Several risk factors can be underlined, such as patients with functional or anatomical abnormalities of the urinary tract, pregnancy, renal disease, indwelling urinary catheters, diabetes or various immunocompromising diseases. More than three episodes of UTIs represent recurrent UTIs during one year or at least two episodes in 6 months. A frequent situation is a positive urine culture in the absence of UTI symptoms, occurring in 1 - 5% of premenopausal females and up to 15 - 50% of the elderly population [38]. It is called asymptomatic bacteriuria and is defined as bacterial growth of more than $10^5$ CFU/mL at urine culture in one sample in men and two consecutive samples in women.

*Escherichia coli* is the most prevalent uropathogenic microorganism in the dynamics of a UTI, counting for almost 57% of the total number of strains. It is followed by *Klebsiella* spp., *Pseudomonas* spp., and *Proteus* spp. in terms of Gram-negative uropathogens. The ubiquitous Gram-positive pathogen is represented by *Enterococcus* spp., followed by *Staphylococcus* spp.; the latter is also less common [16]. Preliminary data suggest that the highest negative trends imply the use of penicillin (R = 33.76%), levofloxacin (R = 27.32), amoxicillin-clavulanic acid (R = 18.16) and ampicillin (R = 14.62%); the most preserved sensitivity was observed for carbapenems - imipenem (S = 95.97%) and meropenem (S = 94.79%), followed by amikacin (S = 91.07%), fosfomycin (S = 88.10%), aztreonam (S = 87.75%) and ceftazidime (S = 86.26%) [44]. This is valuable information for every clinician involved in UTI management.

The urge of extended research involving patients from all three major regions of Romania - Transylvania, Moldova, and Muntenia has raised the interest of a multicenter study. Our results can provide a multivariate image of the microbiological reality of common uropathogens, giving directions to new perspectives in the interest of a modern and efficient treatment of UTIs.

**Materials and Methods**

This four-centre retrospective, cross-sectional study was conducted in tertiary-centre hospitals in three different regions of Romania, as follows: “Prof. Dr. Th. Burghele” Clinical Hospital (BCH) and Elias University Hospital (ECH) in Bucharest, Mures County Clinical Hospital (MCH) and Vaslui County Hospital (VCH). The database has embedded patient results evaluated in each hospital for four months, between 1 September and 31 December 2018. Each of the involved centres conducted the study in accordance with the Declaration of Helsinki; written informed consent was obtained for each patient, and the Ethics Committee approved the protocol of every hospital.

Every study centre followed International Safety Standards [22] for urine collection techniques; sterile receptacles were used for urine collection, followed by culture on lactose agar and Columbia sheep agar. Clinical Laboratory Standards Institute (CLSI) guidelines [54] were used to determine the susceptibility for each bacterial strain for various antimicrobial agents following the disk diffusion technique and antibiograms. The authors previously described the extensive description of the method [6, 46].

A total number of 15,907 patients were evaluated by mid-stream urine cultures, of which 3,011 showed more than $10^5$ CFU/mL. We determined 2,842 subjects from the previous cohort to meet the inclusion criteria in the study, representing 1,690 female patients and 1,152 males. The division of patients into centers was as follows: “Prof. Dr. Th. Burghele” Clinical Hospital - 1045, Elias University Hospital - 981, Vaslui Clinical Hospital - 553 and Mures County Clinical Hospital - 263.

All four centres enrolled both hospitalized and treated ambulatory patients; general information as social-demographic status, sex, and age were taken into account for each patient. More comprehensive information regarding medical history was unable to obtain. The inclusion criteria in the study were represented by: positive urine culture (more than $10^5$ CFU/mL), a single bacteria strain on uroculture, and age over 18 years old. The exclusion criteria were: uroculture with less than $10^5$ CFU/mL, an indwelling catheter, and uroculture with multiple bacterial strains. A visual diagram of the participant patients is represented in Figure 1.

*Statistical analysis*

Data obtained were analysed using Microsoft Excel software (version 2016, Microsoft Corporation, Redmond, WA, USA), and GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA). A p value <0.05 was considered significant.

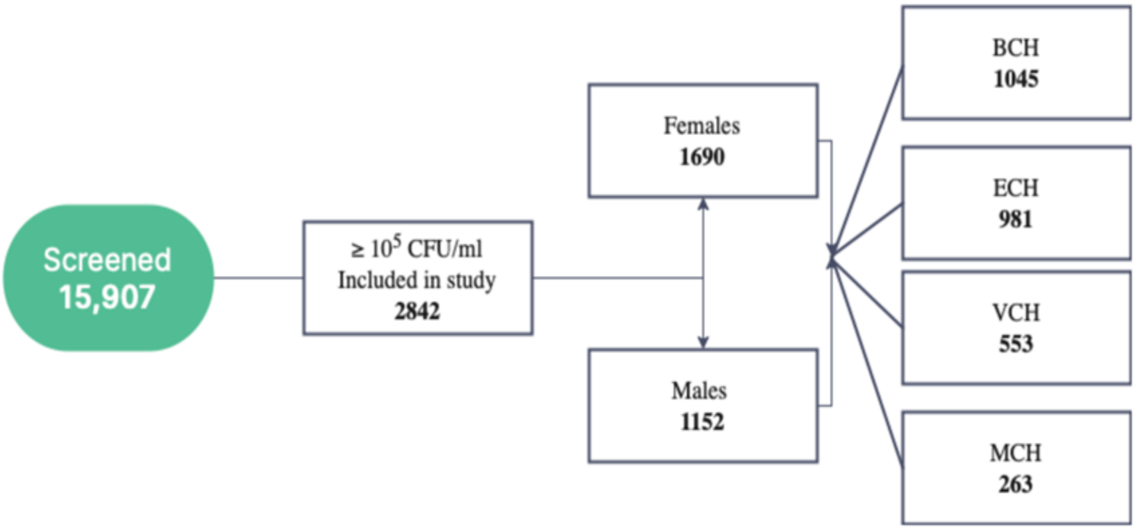


**Figure 1.**
Representative diagram of patients included in the study

**Results and Discussion**

The presented cohort observed an increased incidence of UTIs in female patients compared to the male population with a rise in incidence with almost 50%, representing 1,690 female patients (59.46%), whereas in males, 1,152 (40.53) individuals presenting clinically manifesting UTI. In all patients, it can be observed that incidence rises with each of the evaluated group age; furthermore, in the senior population, the presence of UTIs is more than double compared to the previously noted group age, the middle-age individuals - 35.39% *vs*. 14.53% while in the males 26.74% *vs*. 11.01%. This data is in accordance with the general data on UTIs, considering the direct proportionality between age and incidence, especially in the male population [50], whereas in females, previous studies suggested a rise in the teenagers and young and sexually active women, continuing with a decrease in the 35 - 65 years old group and another surge in the senior population [33, 50]. The higher incidence in the +65 years old group is noted to be in association with multiple general and specific risk factors of the older patients such as urinary obstruction, renal failure, immunosuppression, foreign bodies (calculi), prostate enlargement, tight phimosis, or various neurological diseases [13, 28]. A more detailed representation of the dynamics of UTIs in accordance with age in both male and female populations of the presented study is represented in Table I. *Escherichia coli* is the ubiquitous most present uropathogen involving UTIs in all previous studies; a recent meta-analysis of 90 studies conducted between 1991 and 2015 has shown *E. coli* as the predominant uropathogen, accounting for 62%, followed by *Klebsiella* spp. with 13% [36]. This study has uncovered similarities in terms of epidemiology, showing *E. coli* the frequent bacteria strain representing 57.31% of the total results; the trends are synchronous in both male and female patients. It is followed by *Klebsiella* spp. (18.64%), *Proteus* spp. (5.48%) and *Pseudomonas* spp. (4.22%). Similar data was previously published by various authors [26, 39, 48].

**Table I**

Uropathogens in female and male patients of various age groups

| Age groups (Years) | Female | | Male | |
|---|---|---|---|---|
| | *n* | % | *n* | % |
| **< 30** | 114 | 4.01 | 31 | 1.09 |
| **30 - 45** | 157 | 5.52 | 48 | 1.68 |
| **46 - 65** | 413 | 14.53 | 313 | 11.01 |
| **> 65** | 1006 | 35.39 | 760 | 26.74 |
| **Total** | 1690 | 59.46 | 1152 | 40.53 |

Considering the Gram-positive uropathogens, *Enterococcus* spp. is the leading strain, representing 11.96%, followed by *Staphylococcus* spp. accounting for 2.35% of the total tested probes. Current data is similar to others

[25], even if a recent regional study has diagnosed some strains of *Streptococcus* spp. (especially *S. agalactiae*); the authors admitted that it represents a minority of the cases [18]. Details of the epidemiology of uropathogens involved in the pathology of the tested cohort and its dynamics in the male and female population are comprehensively presented in Table II.

**Table II**

Isolated uropathogens in the study group

| Isolated bacteria | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | *n* | *%* | *n* | *%* | *n* | *%* |
| ***Gram-negative bacteria*** | | | | | | |
| *Escherichia coli* | 485 | 42.1 | 1144 | 67.69 | 1629 | 57.31 |
| *Klebsiella* spp. | 269 | 23.35 | 261 | 15.44 | 530 | 18.64 |
| *Pseudomonas aeruginosa* | 90 | 7.81 | 30 | 1.77 | 120 | 4.22 |
| *Proteus* spp. | 91 | 7.89 | 65 | 3.84 | 156 | 5.48 |
| ***Gram-positive bacteria*** | | | | | | |
| *Enterococcus* spp. | 176 | 15.27 | 164 | 9.7 | 340 | 11.96 |
| *Staphylococcus* spp. | 41 | 3.55 | 26 | 1.53 | 67 | 2.35 |

The burden of general antibiotic resistance had significantly increased during the last decade, especially in pathogens involved in UTIs. A prudent and more justifiable recommendation considering its' treatment is advisable, and prescribing antimicrobials should approach serious consideration, weighting between beneficial and overtreatment. In terms of resistance, this study highlighted the overall results in both male and female patients - *Escherichia coli* with the lowest antimicrobial susceptibility to the two of the most prescribing antibiotics in UTIs: levofloxacin (R = 20.62%) and amoxicillin-clavulanic acid. (R = 18.17%). The trends are similar for both genders: fluoroquinolones (males - R = 23.29%, females - R = 19.49%), as for aminopenicillins (males - R = 25.36%, females - R = 15.12%).

The highest sensitivity to the tested antimicrobials for *Escherichia coli* was observed for fosfomycin S = 91.03% (males - S = 90.72%, females - S = 91.17%), followed by ceftazidime - S = 77.04% (males - S = 59.79%, females - S = 84.35%). High resistance to fluoroquinolones was also observed in regional countries, where the resistance rates also exceed 20%; results were published in a recent study from 2019 involving European countries, including the Eastern region [40]. Back in 2007 in Spain, Gunernado M [20] raised awareness for the high rates of fluoroquinolones resistance, noting that around 18% of the tested strains of *Escherichia coli* were not susceptible to this antimicrobial class. A recent study from 2020 analysing the multidrug resistance of uropathogens has highlighted the alarming increased resistance of *E. coli* to fluoroquinolones. However, the maximum resistance of this particular pathogen was observed to ampicillin and cephalosporins [31]. Another regional study conducted by Falagas ME [11] has shown comparable results to this study, noting around 6% resistance to cephalosporins for *Escherichia coli*. Fortunately, we acknowledge promising results for fosfomycin and nitrofurantoin - overall sensibility for the latter S = 65.56%. They remain the first-line treatment in uncomplicated UTIs in women. Gardiner BJ recently published in 2019 [19] a comparing study involving these two antimicrobials, underling their importance in the preliminary treatment of UTIs before upgrading to other broad-spectrum antibiotics, leading to furthermore rise in the resistance.

*Klebsiella* spp. is the second most common Gram-negative uropathogen and one of the most important determinant bacteria in complicated UTIs; a recent study from 2021 [10] highlighted the rising incidence of this pathogen implicated in the epidemiology of UTIs and its alarming rise in antimicrobial resistance. Moreover, previous papers had shown its implication in public health concerns regarding high mortality rates in nosocomial infections associated with health-care services [8, 47]. This study has underlined alarming resistance for amoxicillin-clavulanic acid R = 45.66% (males - R = 59.85%, females - R = 31.03%), followed by ceftazidime R = 29.05% (males - R = 43.49%, females - R = 14.17%) and levofloxacin R = 24.71% (males - R = 34.57%, females - R = 14.55%). A predominance of higher resistance rates in the male cohort was observed in all cases compared to females for all antimicrobial tested. A previous study from New Delhi in 2018 [49] has shown similarities in terms of resistance for *Klebsiella* spp. for levofloxacin R = 25%, higher rates for ceftazidime R = 75%, and lower resistance for amoxicillin-clavulanic acid R = 25%. Caneiras C *et al*. published a paper in early 2019 in Portugal [5] that followed to determine the differences between community-acquired and hospital-acquired *Klebsiella pneumoniae*; it highlighted the important susceptibility of this pathogen in raising rapid resistance when exposed to a favourable environment in prescribing antibiotics such as hospitals. The study concluded that multidrug-resistant Klebsiella spp. is omnipresent in a hospital environment with serious resistance concerns for various antibiotics classes such as fluoroquinolones and cephalosporins aminopenicillins, and aminoglycosides. We also observed important resistance to amikacin, especially in the male cohort

R = 24.53%. A recent regional mid-summer 2021 report from Iasi, Romania [34] also pointed out the high susceptibility for resistance of *Klebsiella* spp. to the aforementioned classes of antibiotics.

In terms of pathogenicity, *Pseudomonas aeruginosa* is a common pathogen in the dynamics of UTIs and one of the essential microorganisms associated with hospital-acquired and catheter-associated UTIs [35]. Seen as the most frequent pathogen associated with nosocomial infections, it presents an unfavourable prognosis due to polyresistant bacteria [43]. The proposed study highlighted significant resistance to multiple antimicrobial classes: levofloxacin R = 48.33%, ceftazidime R = 38.33%, and amikacin R = 35.83%. Moreover, alarming resistance was also observed for broad-spectrum antimicrobial classes such as carbapenems – meropenem and imipenem R = 29.16%. Similar results were obtained in Hungary in a retrospective analysis over a period of 10 years, claiming 43.78% resistance of *P. aeruginosa* to levofloxacin for male inpatients; however, they observed almost half of the resistance this study provides in terms of ceftazidime (R = 18.81%) and amikacin (R = 18.21%) [17]. In early 2015, a study from Pakistan has reported even higher rates of resistance for some of the tested antibiotics. Their numbers are higher than ours and on a larger cohort - ceftazidime R = 56.1% and fluoroquinolones R = 50%, except for amikacin R = 25.3% and imipenem R = 10.4% [51]. A rise in the resistance of *Pseudomonas* spp. for carbapenems was already noticed worldwide more than ten years ago; a review of the literature from Tanya Strateva and Daniel Yordanov has previously shown in 2009 [52].

*Proteus* spp. is a Gram-negative pathogen, part of the *Enterobacteriaceae* producing urea-inducible urease, causing UTIs, especially in patients with several risk factors such as indwelling catheters, reno-urinary lithiasis, and other structural abnormalities of the urinary tract [7]. It is capable of causing complicated UTIs such as pyelonephritis or even asymptomatic bacteriuria in patients with type 2 diabetes or seniors [32, 42]. In this study, *Proteus* was the third most common Gram-negative uropathogen with important resistance rates to amoxicillin-clavulanic ac. R = 30.12%, followed by trimethoprim/sulfamethoxazole R = 24.35%, levofloxacin R = 17.94% and ceftazidime R = 15.38%. These results are in accordance with other data from literature; in 2013, a study from a tertiary hospital from China [29] had underlined the rapid onset of antimicrobial resistance of *Proteus* spp. to common antibiotics used on a daily basis in the clinic, pointing out high resistance rates to ceftazidime R = 12.5% and fluoroquinolones - ciprofloxacin R = 41.7%, levofloxacin R = 8.3% and alarmingly high resistance to trimethoprim/sulfamethoxazole R = 70.8%. A larger international study [1] that followed resistance for *Proteus* spp. in four hospitals from three European countries - Poland, Sweden, and the Czech Republic has proved increased resistance of this Gram-negative strain to multiple classes of antibiotics. They reported finding significant strains of this pathogen's multidrug-resistant (MDR) variants among cultures; an important number of MDR strains of *Proteus* spp. was also previously described in a Romanian population cohort [45]. Promising results were observed in this study for amikacin – S = 80.0%. A study from Gamal FG *et al*. [15] observing the resistance of various Gram-negative pathogens for aminoglycosides has concluded that only 6.3% of the total *Proteus* spp. strains were resistant to amikacin. The study concluded that the combination between amikacin and ciprofloxacin would raise the sensitivity against this bacterium up to 100% [15].

A more detailed representation of uropathogens resistance patterns in the overall population and the dynamics between female and male cohorts is presented, respectively, in Table III, Table IV, Table V, and Table VI.

**Table III**

Gram-negative uropathogens resistance patterns in the study population

| Antibiotics | ***Escherichia coli*** | | | | ***Klebsiella* spp.** | | | | ***Pseudomonas* spp.** | | | | ***Proteus* spp.** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **R** | | **S** | | **R** | | **S** | | **R** | | **S** | | **R** | | **S** | |
| | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** | ***n*** | ***%*** |
| Amikacin | 67 | 4.11 | 1043 | 64.02 | 87 | 16.41 | 357 | 67.35 | 43 | 35.83 | 71 | 59.16 | 13 | 8.33 | 115 | 73.71 |
| Amoxicillin-clavulanic acid | 296 | 18.17 | 1147 | 70.41 | 242 | 45.66 | 274 | 51.69 | - | - | - | - | 47 | 30.12 | 100 | 64.1 |
| Aztreonam | - | - | - | - | 77 | 14.52 | 142 | 26.79 | 12 | 10.0 | 39 | 32.5 | 8 | 5.12 | 74 | 47.43 |
| Trimethoprim/ sulfamethoxazole | 290 | 17.80 | 812 | 49.84 | 104 | 19.62 | 173 | 32.64 | - | - | - | - | 38 | 24.35 | 31 | 19.87 |
| Ceftazidime | 139 | 8.53 | 1255 | 77.04 | 154 | 29.05 | 353 | 66.60 | 46 | 38.33 | 71 | 59.16 | 24 | 15.38 | 126 | 80.76 |
| Fosfomycin | 21 | 1.28 | 1483 | 91.03 | 20 | 3.77 | 91 | 17.16 | - | - | - | - | - | - | - | - |
| Imipenem | 2 | 0.12 | 473 | 29.03 | 38 | 7.16 | 337 | 63.58 | 35 | 29.16 | 76 | 63.33 | 2 | 1.28 | 82 | 52.56 |
| Levofloxacin*, # | 336 | 20.62 | 1038 | 63.72 | 131 | 24.71 | 348 | 65.66 | 58 | 48.33 | 54 | 45.0 | 28 | 17.94 | 96 | 61.53 |
| Meropenem | 2 | 0.12 | 662 | 40.63 | 49 | 9.24 | 380 | 71.69 | 35 | 29.16 | 80 | 66.66 | 2 | 1.28 | 110 | 70.51 |
| Nitrofurantoin | 95 | 5.83 | 1068 | 65.56 | 78 | 14.71 | 168 | 31.69 | - | - | - | - | - | - | - | - |

R – resistant, S – sensitive, n – number, % – percentage, * $p < 0.05$ when compared resistance profile of levofloxacin and amikacin, # $p < 0.05$ when compared resistance profile of levofloxacin and amoxicillin-clavulanic acid

**Table IV**

Gram-negative uropathogens resistance patterns in female patients in the study population

| Antibiotics | *Escherichia coli* | | | | *Klebsiella* spp. | | | | *Pseudomonas* spp. | | | | *Proteus* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | | S | | R | | S | | R | | S | | R | | S | |
| | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| Amikacin | 40 | 3.49 | 770 | 67.30 | 21 | 8.04 | 186 | 71.26 | 7 | 23.33 | 22 | 73.33 | 5 | 7.69 | 52 | 80.0 |
| Amoxicillin-clavulanic acid | 173 | 15.12 | 910 | 79.54 | 81 | 31.03 | 177 | 67.81 | - | - | - | - | 16 | 24.61 | 45 | 69.23 |
| Aztreonam | - | - | - | - | 15 | 5.74 | 60 | 22.98 | 3 | 10.0 | 11 | 36.66 | 4 | 6.15 | 30 | 46.15 |
| Trimethoprim/ sulfamethoxazole | 205 | 17.91 | 610 | 53.32 | 50 | 19.15 | 108 | 41.37 | - | - | - | - | 12 | 18.46 | 16 | 24.61 |
| Ceftazidime | 76 | 6.64 | 965 | 84.35 | 37 | 14.17 | 209 | 80.07 | 10 | 33.33 | 19 | 63.33 | 10 | 15.38 | 55 | 84.61 |
| Fosfomycin | 11 | 0.96 | 1043 | 91.17 | 7 | 2.68 | 56 | 21.45 | - | - | - | - | - | - | - | - |
| Imipenem | 2 | 0.17 | 282 | 24.65 | 7 | 2.68 | 157 | 60.15 | 8 | 26.66 | 21 | 70.0 | 1 | 1.53 | 34 | 52.3 |
| Levofloxacin*, # | 223 | 19.49 | 798 | 69.75 | 38 | 14.55 | 216 | 82.75 | 10 | 33.33 | 20 | 66.66 | 14 | 21.53 | 43 | 66.15 |
| Meropenem | 2 | 0.17 | 428 | 37.41 | 15 | 5.74 | 180 | 68.96 | 6 | 20.0 | 21 | 70.0 | 1 | 1.53 | 40 | 61.53 |
| Nitrofurantoin | 47 | 4.1 | 731 | 63.89 | 42 | 16.09 | 104 | 39.84 | - | - | - | - | - | - | - | - |

R – resistant, S – sensitive, n – number, % – percentage, * p < 0.05 when compared resistance profile to levofloxacin and amikacin, # p < 0.05 when compared resistance profile to levofloxacin and amoxicillin-clavulanic acid

**Table V**

Gram-negative uropathogens resistance patterns in male patients in the study population

| Antibiotics | *Escherichia coli* | | | | *Klebsiella* spp. | | | | *Pseudomonas* spp. | | | | *Proteus* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | | S | | R | | S | | R | | S | | R | | S | |
| | *n* | *%* | *n* | *%* | *n* | *%* | *n* | *%* | *n* | *%* | *n* | *%* | *n* | *%* | *n* | *%* |
| Amikacin | 27 | 5.56 | 273 | 56.28 | 66 | 24.53 | 171 | 63.56 | 36 | 40.0 | 49 | 54.44 | 8 | 8.79 | 63 | 69.23 |
| Amoxicillin-clavulanic acid | 123 | 25.36 | 237 | 48.86 | 161 | 59.85 | 97 | 36.05 | - | - | - | - | 31 | 34.06 | 55 | 60.43 |
| Aztreonam | - | - | - | - | 62 | 23.04 | 82 | 30.48 | 9 | 10.0 | 28 | 31.11 | 4 | 4.39 | 44 | 48.35 |
| Trimethoprim/ sulfamethoxazole | 85 | 17.52 | 202 | 41.64 | 54 | 20.07 | 65 | 24.16 | - | - | - | - | 26 | 28.57 | 15 | 16.48 |
| Ceftazidime | 63 | 12.98 | 290 | 59.79 | 117 | 43.49 | 144 | 53.53 | 36 | 40.0 | 52 | 57.77 | 14 | 15.38 | 71 | 78.02 |
| Fosfomycin | 10 | 2.06 | 440 | 90.72 | 13 | 4.83 | 35 | 13.01 | - | - | - | - | - | - | - | - |
| Imipenem | 0 | 0 | 191 | 39.38 | 31 | 11.52 | 180 | 66.91 | 27 | 30.0 | 55 | 61.11 | 1 | 1.09 | 48 | 52.74 |
| Levofloxacin*, # | 113 | 23.29 | 240 | 49.48 | 93 | 34.57 | 132 | 49.07 | 48 | 53.33 | 34 | 37.77 | 14 | 15.38 | 53 | 58.24 |
| Meropenem | 0 | 0 | 234 | 48.24 | 34 | 12.63 | 200 | 74.34 | 29 | 32.22 | 59 | 65.55 | 1 | 1.09 | 70 | 76.92 |
| Nitrofurantoin | 50 | 10.3 | 337 | 69.48 | 36 | 13.38 | 64 | 23.79 | - | - | - | - | - | - | - | - |

R – resistant, S – sensitive, n – number, % – percentage, * p < 0.05 when compared resistance profile to levofloxacin and amikacin, # p < 0.05 when compared resistance profile to levofloxacin and amoxicillin-clavulanic acid

**Table VI**

Gram-positive uropathogens resistance patterns in the study population

| Antibiotics | *Enterococcus* spp. | | | | *Staphylococcus* spp. | | | |
|---|---|---|---|---|---|---|---|---|
| | R | | S | | R | | S | |
| | n | % | n | % | n | % | n | % |
| Amikacin | - | - | - | - | 2 | 2.98 | 50 | 74.62 |
| Ampicillin | 55 | 16.17 | 266 | 78.23 | - | - | - | - |
| Trimethoprim/sulfamethoxazole | - | - | - | - | 21 | 31.34 | 35 | 52.23 |
| Ceftazidime | - | - | - | - | 8 | 11.94 | 40 | 59.70 |
| Fosfomycin | 8 | 2.35 | 214 | 62.94 | - | - | - | - |
| Levofloxacin | 110 | 32.35 | 167 | 49.11 | 20 | 29.85 | 39 | 58.20 |
| Linezolid | 6 | 1.76 | 251 | 73.82 | 2 | 2.98 | 46 | 68.65 |
| Nitrofurantoin | 13 | 3.82 | 254 | 74.70 | 0 | 0 | 45 | 67.71 |
| Penicillin | 86 | 25.29 | 172 | 50.58 | 35 | 52.23 | 14 | 20.89 |
| Vancomycin | 5 | 1.47 | 269 | 79.11 | - | - | - | - |

*Enterococcus* spp. is the most frequent Gram-positive strain involved in the pathology of UTIs. It is physiologically part of the microflora of the human gastrointestinal tract and may temporarily pathologically inoculate the urinary tract. It is a microorganism inherently resistant to aminoglycosides, cephalosporins, and *in vivo* to the combination of trimethoprim/sulfamethoxazole [37]. In our study, significant resistance was observed for levofloxacin R = 32.35% and penicillin R = 25.29 and ampicillin

R = 16.17%; the highest sensitivity rates were observed for vancomycin S = 79.11%, followed by nitrofurantoin S = 74.70%, linezolid S = 73.82%. A relative resistance was observed for fosfomycin S = 62.94%. A large study from India [21] published in 2016 that followed and screened over 22,800 urine cultures has shown better results than ours in terms of fosfomycin and linezolid, acquiring 0% resistance rates, but a poor outcome for ampicillin R = 36.1% and vancomycin R = 22.9%. Similar results were reported in a study from Germany regarding ampicillin R = 15% [23]. Linezolid, a second line treatment for *Enterococcus* spp., displayed good results. This data must be treated carefully as a recent study from 2021 in China [30] has determined a large number of linezolid resistant and intermediate-resistant strains to this particular drug, raising awareness of the hospital-acquired mechanism of resistance.

The second most common Gram-positive uropathogen is represented by *Staphylococcus* spp. It is an opportunistic microorganism affecting both immune-competent and compromised patients, generally resulting in high morbidity and serious complications, burdening the health care systems [27]. *Staphylococcus* spp. is known to rapidly increase resistance to current treatments and the new drugs [2, 55]. We report the highest resistance of this bacterium to penicillin R = 52.23%, followed by trimethoprim/sulfamethoxazole R = 31.34% and levofloxacin R = 29.85%. The highest sensitivity was achieved for amikacin R = 74.62%, linezolid R = 68.65%, and nitrofurantoin R = 67.71%. A study from Nigeria developed by Adebola Onanuga in 2012 [41] presented higher resistance to the tested antibiotics: ampicillin R = 100%, aminoglycosides R = 73.9%, and nitrofurantoin R = 39.1%. A meta-analysis from Serawit Deyno published in summer 2017 [9] has shown increased resistance to multiple antimicrobial classes, such as ampicillin R = 75%, trimethoprim/ sulfamethoxazole R = 47%, ciprofloxacin R = 19%, and vancomycin R = 11%. They concluded that *Staphylococcus* spp. had gained serious antimicrobial resistance to almost all antibiotics used.

In terms of limitations, one of the most crucial issues of this study is represented by the limited number of studied patients – the more patients included in the study, the more accurate the results. Another limitation of the present work is the lack of a comparison group of study from the same geographical area, so it could be determined if the current resistance patterns are evolving; further studies are needed to unravel this trend. Lastly, more detailed history for each patient isn't available to mention whether it is the first episode of UTI or a recurrent infection previously treated with antibiotics, whether patients had a history of indwelling catheters or not, or if patients presented symptoms of UTI at presentation.

**Conclusions**

UTIs represent a serious health problem where antibiotic treatment is a key factor in combating this pathology; prescribing potent antimicrobial has increased antibiotic resistance, burdening healthcare systems. The current data have shown *Escherichia coli* representing the ubiquitous pathogen involved, followed by *Klebsiella spp*. as the second most common Gram-negative pathogen. Alarming resistance was observed for two of the most prescribed antibiotics in managing UTIs: levofloxacin and amoxicillin-clavulanic ac. *Enterococcus* spp. is the most frequent Gram-positive bacteria involved, with leading resistance to levofloxacin, ampicillin, and penicillin.

Hence, judicious management of antibiotic treatment is an essential tool in limiting the evolution of resistance. One general guideline is not satisfactory, as adapting antibiotic treatment to local resistance patterns provides better results; thus, this study combining results from all three major regions of Romania is of utmost importance in the treatment orientation for every clinician managing UTIs.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


1. Adamus-Bialek W, Zajac E, Parniewski P, Kaca W. Comparison of antibiotic resistance patterns in collections of *Escherichia coli* and *Proteus mirabilis* uropathogenic strains. *Mol Biol Rep.*, 2013; 40(4): 3429-3435.
2. Akortha EE, Ibadin OK, Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection (UTIS) in UBTH Benin City, Nigeria. *Afr J Biotechnol.*, 2008; 7: 1637-1640.
3. Alós, JI, Epidemiología y etiología de la infección urinaria comunitaria. Sensibilidad antimicrobiana de los principales patógenos y significado clínico de la resistencia. *Enferm Infecc Microbiol Clin.*, 2005; 23(Suppl 1): 3-8, (available in Spanish).
4. Bonkat G, Bartoletti R, Bruyère F, Cai T, Geerlings SE, Köves B, Schubert S, Wagenlehner F, EAU Guidelines on Urological Infections, European Association of Urology: Arnhem, The Netherlands, 2021.
5. Caneiras C, Lito L, Melo-Cristino J, Duarte A, Community- and hospital-acquired *Klebsiella pneumoniae* urinary tract infections in Portugal: virulence and antibiotic resistance. *Microorganisms*, 2019; 7(5): 138: 1-14.
6. Chibelean CB, Petca RC, Mares C, Popescu RI, Eniko B, Mehedintu C, Petca A, A clinical perspective on the antimicrobial resistance spectrum of uropathogens in a Romanian male population. *Microorganisms*, 2020; 8(6): 848: 1-15.
7. Coker C, Poore CA, Li X, Mobley HL, Pathogenesis of Proteus mirabilis urinary tract infection. *Microbes Infect.*, 2000; 2(12): 1497-1505.

8. Davarcı İ, Şenbayrak S, Aksaray S, Koçoğlu ME, Kuşkucu MA, Samastı M, Molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* isolates. *Anadolu Klin.*, 2019; 24(1): 1-7.
9. Deyno S, Fekadu S, Astatkie A, Resistance of *Staphylococcus aureus* to antimicrobial agents in Ethiopia: a meta-analysis. *Antimicrob Resist Infect Control.*, 2017; 6: 85.
10. Ding Y, Wang H, Pu S, Huang S, Niu S, Resistance trends of *Klebsiella pneumoniae* causing urinary tract infections in Chongqing, 2011-2019. *Infect Drug Resist.*, 2021; 14: 475-481.
11. Falagas ME, Polemis M, Alexiou VG, Marini-Mastrogiannaki A, Kremastinou J, Vatopoulos AC, Antimicrobial resistance of *Escherichia coli* urinary isolates from primary care patients in Greece. *Med Sci Monit.*, 2008; 14(2): CR75-79.
12. Foxman B, Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med.*, 2002; 113(Suppl 1A): 5S-13S.
13. Foxman B, Recurring urinary tract infection: Incidence and risk factors. *Am. J. Public Health*, 1990; 80(3): 331-333.
14. Foxman B, Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infect Dis Clin North Am.*, 2014; 28(1): 1-13.
15. Gad GF, Mohamed HA, Ashour HM, Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. *PLoS One*, 2011; 6(2): e17224: 1-7.
16. Gajdács M, Ábrók M, Lázár A, Burián K, Comparative epidemiology and resistance trends of common urinary pathogens in a tertiary-care hospital: a 10-year surveillance study. *Medicina (Kaunas).*, 2019; 55(7): 356: 1-15.
17. Gajdács M, Ábrók M, Lázár A, Burián K, Epidemiology and antibiotic resistance profile of bacterial uropathogens in male patients: A 10-year retrospective study. *Farmacia*, 2021; 69(3): 530-539.
18. Gajdács M, Ábrók M, Lázár A, Burián K, Increasing relevance of Gram-positive cocci in urinary tract infections: a 10-year analysis of their prevalence and resistance trends. *Sci Rep.*, 2020; 10(1): 17658: 1-11.
19. Gardiner BJ, Stewardson AJ, Abbott IJ, Peleg AY, Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. *Aust Prescr.*, 2019; 42(1): 14-19.
20. Gobernado M, Valdés L, Alós JI, García-Rey C, Dal-Ré R, García-de-Lomas J; Spanish Surveillance Group for urinary pathogens, antimicrobial susceptibility of clinical *Escherichia coli* isolates from uncomplicated cystitis in women over a 1-year period in Spain. *Rev Esp Quimioter.*, 2007; 20(1): 68-76.
21. Goel V, Kumar D, Kumar R, Mathur P, Singh S, Community acquired *Enterococcal* urinary tract infections and antibiotic resistance profile in north India. *J Lab Physicians.*, 2016; 8(1): 50-54.
22. Guidelines for the Collection of Clinical Specimens during Field Investigation of Outbreaks; World Health Organization: Geneva, Switzerland, 2000; pp. 1-51.
23. Haller M, Brandis M, Berner R, Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. *Pediatr Nephrol.*, 2004, 19(9): 982-986.
24. Harding GK, Ronald AR, The management of urinary infections: what have we learned in the past decade?. *Int J Antimicrob Agents.*, 1994; 4(2): 83-88.
25. Kline KA, Lewis AL, Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Microbiol Spectr.*, 2016; 4(2): 1-54.
26. Kline KA, Schwartz DJ, Lewis WG, Hultgren SJ, Lewis AL, Immune activation and suppression by group B streptococcus in a murine model of urinary tract infection. *Infect Immun.*, 2011; 79(9): 3588-3595.
27. Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, Killgore G, Tenover FC, Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. *J Infect Dis.*, 2006; 193(2): 172-179.
28. Levison ME, Kaye D, Treatment of complicated urinary tract infections with an emphasis on drug-resistant Gram-negative uropathogens. *Curr Infect Dis Rep.*, 2013; 15(2): 109-115.
29. Ma KL, Wang CX, Analysis of the spectrum and antibiotic resistance of uropathogens in vitro: results based on a retrospective study from a tertiary hospital. *Am J Infect Control.*, 2013; 41(7): 601-606.
30. Ma X, Zhang F, Bai B, Lin Z, Xu G, Chen Z, Sun X, Zheng J, Deng Q, Yu Z, Linezolid resistance in *Enterococcus faecalis* associated with urinary tract infections of patients in a tertiary hospital in China: resistance mechanisms, virulence, and risk factors. *Front Public Health.*, 2021; 9: 570650: 1-8.
31. Manciuc C, Mihai IF, Filip-Ciubotaru F, Lacatusu GA, Resistance profile of multidrug-resistant urinary tract infections and their susceptibility to carbapenems. *Farmacia*, 2020; 68(4): 715-721.
32. Matthews SJ, Lancaster JW, Urinary tract infections in the elderly population. *Am J Geriatr Pharmacother.*, 2011; 9(5): 286-309.
33. Medina M, Castillo-Pino E, An introduction to the epidemiology and burden of urinary tract infections. *Ther Adv Urol.*, 2019; 11(3-7): 1-5.
34. Miftode IL, Nastase EV, Miftode RȘ, Miftode EG, Iancu LS, Luncă C, Anton Păduraru DT, Costache II, Stafie CS, Dorneanu OS, Insights into multidrug-resistant *K. pneumoniae* urinary tract infections: from susceptibility to mortality. *Exp Ther Med.*, 2021; 22(4): 1086: 1-9.
35. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K, Urinary tract infections caused by *Pseudomonas aeruginosa:* a minireview. *J Infect Public Health.*, 2009; 2(3): 101-111.
36. Mortazavi-Tabatabaei SAR, Ghaderkhani J, Nazari A, Sayehmiri K, Sayehmiri F, Pakzad I, Pattern of antibacterial resistance in urinary tract infections: a systematic review and meta-analysis. *Int J Prev Med.*, 2019; 10: 169: 1-16.
37. Murray BE, The life and times of the *Enterococcus*. *Clin Microbiol Rev.*, 1990; 3(1): 46-65.
38. Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM, Infectious Diseases Society of America, American Society of Nephrology, American Geriatric

Society, Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis.*, 2005; 40(5): 643-654.
39. Nielubowicz GR, Mobley HL, Host–pathogen interactions in urinary tract infection. *Nature Rev Urol.*, 2010; 7(8): 430-441.
40. Ny S, Edquist P, Dumpis U, Gröndahl-Yli-Hannuksela K, Hermes J, Kling AM, Klingeberg A, Kozlov R, Källman O, Lis DO, Pomorska-Wesołowska M, Saule M, Wisell KT, Vuopio J, Palagin I; NoDARS UTIStudy Group, Antimicrobial resistance of Escherichia coli isolates from outpatient urinary tract infections in women in six European countries including Russia. *J Glob Antimicrob Resist.*, 2019; 17: 25-34.
41. Onanuga A, Awhowho GO, Antimicrobial resistance of Staphylococcus aureus strains from patients with urinary tract infections in Yenagoa, Nigeria. *J Pharm Bioallied Sci.*, 2012; 4(3): 226-230.
42. Papazafiropoulou A, Daniil I, Sotiropoulos A, Balampani E, Kokolaki A, Bousboulas S, Konstantopoulou S, Skliros E, Petropoulou D, Pappas S, Prevalence of asymptomatic bacteriuria in type 2 diabetic subjects with and without microalbuminuria. *BMC Res Notes.*, 2010; 3: 169: 1-5.
43. Paz-Zarza VM, Mangwani-Mordani S, Martínez-Maldonado A, Álvarez-Hernández D, Solano-Gálvez SG, Vázquez-López R, *Pseudomonas aeruginosa*: Pathogenicity and antimicrobial resistance in urinary tract infection. *Rev Chilena Infectol.*, 2019; 36(2): 180-189, (available in Spanish).
44. Petca RC, Mareș C, Petca A, Negoiță S, Popescu RI, Boț M, Barabás E, Chibelean CB, Spectrum and antibiotic resistance of uropathogens in Romanian females. *Antibiotics*, 2020; 9(8): 472: 1-16.
45. Petca RC, Negoiță S, Mareș C, Petca A, Popescu RI, Chibelean CB, Heterogeneity of antibiotics multidrug-resistance profile of uropathogens in Romanian population. *Antibiotics*, 2021; 10(5): 523: 1-13.
46. Petca RC, Popescu RI, Mares C, Petca A, Mehedintu C, Sandu I, Maru N, Antibiotic resistance profile of common uropathogens implicated in urinary tract infections in Romania. *Farmacia*, 2019; 67(6): 994-1004.
47. Pitout JDD, Nordmann P, Poirel L, Carbapenemase-producing *Klebsiella pneumoniae*, a key pathogen set for global nosocomial dominance. *Antimicrob Agents Chemother.*, 2015; 59(10): 5873-5884.
48. Pricop C, Jinga V, Puia D, Proca TM, Ivănuță M, Rădăvoi GD, Comparative research between solifenacintamsulosin *versus* tamsulosin alone in expulsion of juxtavesical ureteral stones. *Farmacia*, 2022, 70(4): 652-657.
49. Rizwan M, Akhtar M, Najmi AK, Singh K, *Escherichia coli* and *Klebsiella pneumoniae* sensitivity/resistance pattern towards antimicrobial agents in primary and simple urinary tract infection patients visiting University Hospital of Jamia Hamdard New Delhi. *Drug Res. (Stuttg)*, 2018; 68(7): 415-420.
50. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E, The diagnosis of urinary tract infection: a systematic review. *Dtsch Arztebl Int.*, 2010; 107(21): 361-367.
51. Shah DA, Wasim S, Essa Abdullah F, Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from urine samples of urinary tract infections patients in Karachi, Pakistan. *Pak J Med Sci.*, 2015; 31(2): 341-345.
52. Strateva T, Yordanov D, *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. *J Med Microbiol.*, 2009; 58(Pt 9): 1133-1148.
53. Tan CW, Chlebicki MP, Urinary tract infections in adults. *Singapore Med J.*, 2016; 57(9): 485-490.
54. The Clinical & Laboratory Standards Institute (CLSI). https://clsi.org.
55. Umolu PI, Okoli EN, Izomoh IM, Antibiogram and Beta-lactamases production of *Staphylococcus aureus* isolates from different human clinical specimens in Edo state, Nigeria. *West Afr J Med.*, 2002; 2: 124-127.