https://doi.org/10.31925/farmacia.2019.1.23 **ORIGINAL ARTICLE**

MICROBIOLOGICAL PROFILE AND ANTIBIOTIC RESISTANCE PATTERN OF BACTERIAL UROPATHOGENS AMONG HOSPITALIZED PATIENTS

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Abstract

Urinary tract infection (UTI) is the second most common type of infection and it may be a cause of considerable morbidity in case of recurrence. The aim of this retrospective study was to determine the distribution of pathogens involved in UTIs in hospitalized patients and their drug resistant pattern. It was analysed the distribution of the uropathogens from 427 urine samples collected from hospitalized patients from the urology clinic of County Emergency Clinical Hospital Craiova, between January to December 2017. Identification of the bacterial strains was performed on Chromagar (Oxoid) and the Vitek 2 Compact systems. The most common causative pathogens in UTIs were *E. coli* (45.35%), followed by *Klebsiella spp*., *Enterococcus spp*., *Enterobacter spp*. (8.18%). The strains of *Enterobacteriaceae* presented a high resistance to second and third generation cephalosporin, fluoroquinolones, and amoxicillin-clavulanic-acid. Surveillance of antibiotic prescription and monitoring studies are required to reduce the risk of drug resistance in bacterial uropathogens.

Rezumat

Infecția tractului urinar (ITU) este al doilea cel mai frecvent tip de infecție și poate fi o cauză de morbiditate considerabilă în cazul recidivei. Scopul acestui studiu retrospectiv a fost de a determina distribuția agenților patogeni implicați în ITU la pacienții spitalizați și profilul rezistenței lor la antibiotice. A fost analizată distribuția bacteriilor uropatogene izolate din 427 eșantioane de urină recoltate de la pacienții spitalizați în clinica de Urologie a Spitalului Clinic Județean de Urgență Craiova, în perioada ianuarie-decembrie 2017. Identificarea tulpinilor bacteriene s-a făcut pe mediul chromagar (Oxoid) și pe sistemul Vitek 2 Compact. Cei mai frecvenți agenți patogeni cauzali ai ITU au fost *E. coli* (45,35%), urmată de *Klebsiella spp*., *Enterococcus spp*., *Enterobacter spp*. (8,18%). Tulpinile de *Enterobacteriaceae* au prezentat o rezistență ridicată la cefalosporine de a doua și a treia generație, fluoroquinolone și amoxicilină-acid clavulanic. Supravegherea studiilor privind prescrierea antibioticelor și studiile de monitorizare sunt necesare pentru a reduce riscul de rezistență la medicamente al bacteriilor uropatogene.

Keywords: urinary tract infections, strains distribution, antibiotic resistance

Introduction

Urinary tract infection (UTI) is the second most common type of infection and it may be a cause of considerable morbidity in case of recurrence. UTIs are the most common nosocomial infections in both acute-care hospitals and long-term-care facilities [11]. Over 95% of UTIs are caused by *Enterobacteriaceae* from which the most important is *E. coli*, responsible for 90% of the UTI in the non-hospitalized and 50% hospitalized patients [8]. Other bacterial strains causing UTIs include *Klebsiella*, *Proteus*, *Pseudomonas*, *Enterococcus*, *Enterobacter* spp. etc. Organisms such as *Serratia* and *Pseudomonas* assume increasing importance in recurrent infections and infections associated with urologic obstructions. They also play a major role in nosocomial and catheter associated infections [1]. Among the risk factors for UTI are also gender, immune deficiencies, diabetes, chronic digestive disorders, associated genital disorders, prostatic or neurological disorders, obstructions in the urinary tract [8]. The emergence and widespread of antimicrobial resistance (AMR) is now considered a global public health threat [20]. Recurrent UTI are associated with bladder cancer [28] and might interfere the imunohistological differential diagnosis [22]. Starting from this reality, we analyzed the distribution and resistance patterns of the uropathogens to different drugs commonly used to treat urinary infections in hospitalized patients.

Materials and Methods

The study included the determination of pathogens involved in UTIs in patients admitted to the urology clinic of County Emergency Clinical Hospital Craiova, Romania, a county hospital with 1,518

beds, which provides healthcare to patients from Dolj county and Oltenia region. Data were collected from January 2017 to December 2017 from the clinical pathology databases of the hospital, including reports of technical procedures conducted and urine culture tests performed in the laboratory.

We analysed the resistance patterns for the action of the appropriate antibiotics for the isolated strains of urine specimens in the hospital's laboratory. Identification of the bacterial strains in the selected samples for this study was performed on Chromagar (Oxoid) and the Vitek 2 Compact system. A specimen was considered positive for UTI with a number of yielded colonies $\geq 10^5$ CFU/mL.

Antibiotic testing was carried out using the Vitek 2 Compact system and the diffusion method. Antibiotics agents employed for susceptibility testing were ampicillin-clavulanic acid (20/10 μg), cefazolin (30 μg), cefuroxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), vancomicyn (30 μg), teicoplanin (30 μg), piperacillin-tazobactam (30 μg), imipenem (10 μg), linezolid (30 μg), tetracycline (30 μg), penicillin (10 μg), erytromycin (15 μg), clindamycin (2 μg), clarithromycin (15 μg), doxycycline (30 μg), oxacillin (1 μg) and moxifloxacin (5 μg). Interpretation was done according to Clinical Laboratory Standard Institute (CLSI) guidelines [23]. Chi-square test was performed to evaluate antibiotic resistance patterns of bacteria. Differences were considered significant when $p \leq 0.05$.

Results and Discussion

Between January and December 2017, there were analysed 427 samples from patients with urological diseases. The mean age of patients was 66±9.89 years, 191 women (44.73%) and 236 men (55.26%) (Table I). Distribution of subjects by age group reflects the largest proportion of elderly patients (≥65 years –47.54%), followed by the average age population (35-64 years– 33.72%), young people (15-34 years -10.3%) and children (0-14 ani– 8.43%). Elderly patients (≥65 years) accounted for almost half (47.54%) of total number of UTI cases, followed by age group 35-64 years (33.72%) and 15-34 years (10.3%). 8.43% from UTI cases were pediatric patients (0-14 years).

A total of 452 bacterial isolates were recovered, of which 384 (84.95%) of the isolates were Gram negative and 68 isolates (15,04%) were Gram positive bacteria.

Table I

Distribution of study subjects regarding gender and age

		Age group	No	%
Gender	**Female**	≤14 years	17	8.90
		15-34 years	29	15.18
		35-64 years	66	35.55
		≥65 years	79	41.36
		Total	191	100
	Male	≤14 years	19	8.05
		15-34 years	15	6.35
		35-64 years	78	33.05
		≥65 years	124	52.54
		Total	236	100
Total subjects		≤14 years	36	8.43
		15-34 years	44	10.3
		35-64 years	144	33.72
		≥65 years	203	47.54
		Total	427	100

The most frequent isolate of the Gram negative uropathogens was *E. coli* (45.35%), followed by *Klebsiella spp*. (20.79%), *Enterococcus spp*. (9.73%), *Enterobacter spp*. (8.18%), *Proteus spp*. (5.30%), *Pseudomonas spp*. (3.09%), *Citrobacter spp* (1.32%), Glucose-nonfermenting Gram-negative bacilli (NFB) (0.88%).

Table II

Microorganisms isolated from urine samples from hospitalized patients in Urology clinic of the County Emergency Clinical Hospital Craiova, Romania, between January-December 2017

Microorganism	Females		Males		Total	
	n	%	n	%	N	%
Escherichia coli	94	47.23	111	43.87	205	45.35
Proteus mirabilis	11	5.52	13	5.13	24	5.30
Klebsiella spp	42	21.10	52	20.55	94	20.79
Citrobacter spp	3	1.50	3	1.18	6	1.32
Enterobacter spp	12	6.03	25	9.88	37	8.18
Pseudomonas aeruginosa	4	28.57	10	71.42	14	3.09

Microorganism	Females		Males		Total	
	n	%	n	%	N	%
MRSA	10	5.02	10	3.95	20	4.42
Enterococcus spp	22	11.05	22	8.69	44	9.73
CoNS	0	0	3	1.18	3	0.66
NFB	1	0.50	3	1.18	4	0.88
Gram positive bacilli	0	0	1	0.39	1	0.22
Total	199	100	253	100	452	100

MRSA - Methicillin-Resistant Staphylococcus Aureus; NFB-Glucose-non-fermenting Gram-negative bacilli*; CoNS* -Coagulase-negative staphylococci

Other investigators have also reported the same first two uropathogens involved in UTIs. The frequency of *Escherichia coli* in urine samples, which is the most frequent cause of bloodstream and urinary tract infections in Europe, varies in different studies [2]. According to our study, *E. coli* and *Klebsiella spp*. detained 66% of the total isolates, representing first and second Gram negative strains, while *Enterococcus spp.* and *Methicillin-Resistant Staphylococcus aureus* (MRSA) were the first and second predominant Gram positive bacteria, accounting for 14.15% from all isolates.

Differences in isolation rates of *E. coli, Klebsiella spp., Proteus spp., Enterobacter spp., Pseudomonas spp*. were observed between males and females. Referring to the total number of samples collected by gender, isolation rates indicates a higher value for female patients for *E.coli* (47.23% compared to 43.87%), *Klebsiella spp.* (21.10% compared to 20.55%), *Enterococcus spp*. (11.05% compared to 8.69%), MRSA (5.02% compared to 3.95%). Instead, other microorganisms were more frequently responsible for UTIs in males than in females, such as *Enterobacter spp*. (9.88% compared to 6.03%) and Pseudomonas aeruginosa, with an almost double value (3.95% compared to 2.01%).

Most strains of *E. coli* were isolated in subjects over 65 years of age (42.55% in women and 53.47% in men, the difference being not statistically significant), followed by the age group 35-64 years (34.04% in women and 36.43% in men). The percentage was lower than that reported in other study [9, 10]. 15.95% of the *E. coli* strains were isolated from urine samples collected from female patients aged 15-34 years and 6.30% from male patients of the same age, the difference being statistically significant ($\chi 2 = 4.94$, $p = 0.02$).

45.23% of the *Klebsiella spp*. strains were isolated in women over 65 years and 55.76% in males over 65 years of age, with only 3 cases being recorded in children ≤ 14 years.

The third most involved uropathogen according to our study was *Enterococcus spp*., representing almost 10%. More than half of the strains of *Enterococcus spp*. were isolated in men aged 35-64 years, while in females, the largest percentage (45.45%) was found in elderly people (≥ 65 years) . This frequency is close to that evidenced by the results of another study conducted in Romania on a group of 85 elderly outpatients [10]. Vallejo-Torres L *et al*. revealed the presence of *Enterococcus spp*. in a percentage of 5% in a study evaluating the costs of hospitalised patients due to complicated urinary tract infections in 20 hospitals from 8 countries including Romania, with high prevalence of multidrug-resistant Gram-negative bacteria. [27] In other studies the third place after *E. coli* and *Klebsiella spp*. was occupied by coagulase-negative staphylococci (CoNS) [25], *Proteus spp*. [14] or NFB [5]. In other studies, the most common pathogens other than *E.coli*, involved in UTIs, were, *Staphylococcus aureus* or *Staphylococcus saprophyticus* [12].

Antimicrobial resistance (AMR) is a serious threat to public health and patient safety in Europe, leading to increasing healthcare costs, patient treatment failure, and deaths. [2] Several classes of bacteria have already exhibited multidrug resistance to antibiotics, such as *Klebsiella pneumoniae* and *E. coli* strains producing extended-spectrum beta-lactamase (ESBL), which hydrolyses the beta-lactam ring of penicillin, cephalosporins, and other related antibiotics, contributing to treatment failure [7, 26].

While antibiotics are considered the most effective method of treatment for bacterial infections, their empirical, indiscriminate, prolonged, or incorrect usage contributes significantly to the emergence of new infections by leading to the selection of resistant strains [15, 17]. For instance, 20% of relapses and 3.7% of new cases of tuberculosis are caused by rifampicin- and isoniazid-resistant strains of *Mycobacterium tuberculosis* [29]. The migration phenomenon contributes to the increased multidrug resistance stains [4].

In the case of urinary (TB), the kidney is the most important site of extra-pulmonary TB [8]. Extrapulmonary tuberculosis includes all TB affects except pleura, lungs, intra-thoracic lymph nodes and larynx, and originates from the hematogenous metastatic affects developed during the prime TB infection period [21].

The evolution of this type of tuberculosis may be early, before the primary infection has healed or long after the primary infection by reactivation of healed lesions. [18] Cutaneous tuberculosis represents a small percent of total extrapulmonary TB forms, caused mainly by *Mycobacterium tuberculosis*. The form of the disease depends on the virulence of the strain, the immune status of the host, the portal of entry, the internal spread pattern, and the adequacy

of the initial treatment [19] and is extremely important in countries with both high and low TB incidence [3, 4].

Fungal UTIs occur in urinary catheterized patients, people receiving broad-spectrum antibiotics or immunosuppressed patients. [8]

The present study revealed that uropathogens isolated from urine samples of patients with UTIs presented high levels of single and multiple antimicrobial resistance.

The antibiotic resistance rates of the isolates are summarized in figure 1. Over 70% from *Enterobacteriaceae* were resistant to ampicillin-clavulanic acid, cefazolin, cefuroxime, while half of them were susceptible to ceftriaxone, 63% to cefepime, 60.18% to piperacillin-tazobactam and almost all to imipenem (93.87%). Regarding fluoroquinolones, 69.12% of *Enterobacteriaceae* were resistant to ciprofloxacin.

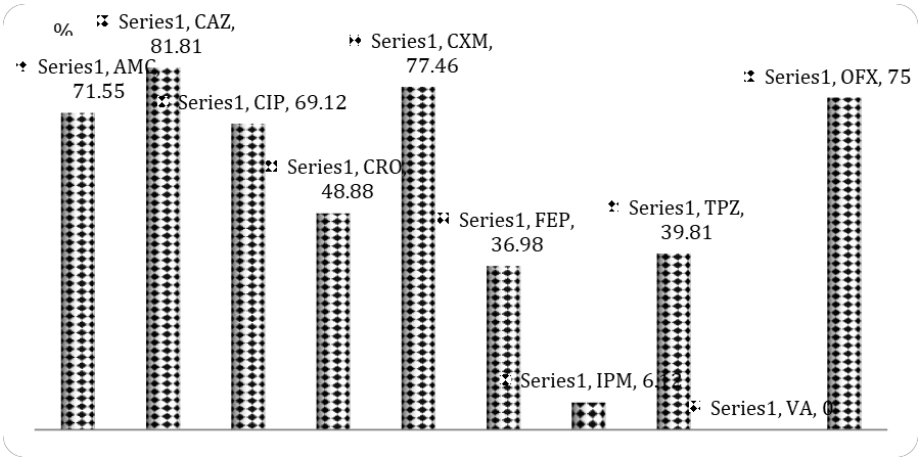

Figure 1

Resistance pattern of *Enterobacteriaceae* to the tested antibiotics. AMC- ampicillin-clavulanic acid; CAZ- ceftazidime ; CIP – ciprofloxacin; CRO - ceftriaxone; CXM- cefuroxime; FEP- cefepime; IPM - imipenem; TPZ - piperacillin-tazobactam; VA- vancomicyn (only *Enterobacter spp*.); OFX – ofloxacin (only *Enterobacter spp*.).

Around 65% of *E. coli* isolates were resistant to ampicillin-clavulanic acid and ciprofloxacin and almost 75% to cefuroxime. The strains tested were almost entirely susceptible to imipenem, 64.5% to ceftriaxone and 72.48% to cefepime. The results are consistent with analyses from the European Centre for Disease Prevention and Control [2].

Another study reflected more than 20% prevalence of resistance among *E. coli* and all isolates combined for ampicillin, cephalothin, and sulfamethoxazole [12], while other researchers have found that 100% of *E. coli* and *K. pneumonia* isolates were resistant to amoxicillin and ampicillin [6], or a high susceptibility to ciprofloxacin [16].

According to the Annual report of the European Antimicrobial Resistance Surveillance Network, the highest resistance percentages in *E. coli* were generally reported from southern and south-eastern Europe [2].

Combined resistance to multiple antimicrobial groups was observed for *Klebsiella spp*., consistent with European Centre for Disease Prevention and control (ECDC). Almost all strains of *Klebsiella spp*. (92.95%) were resistant to ampicillin-clavulanic acid, about 85% at cefuroxime, 73.11% at ciprofloxacin, and nearly 70% at ceftazidime. Around 93% of the tested strains were sensitive to imipenem and 61% to cefepime. Although ECDC analysis places Romania between the three countries with the highest carbapenem resistance [2], in the present study the susceptibility rate for the tested *Klebsiella spp*. strains was 92.85%.

The majority of infections caused by *K. pneumoniae* are healthcare-associated and the most common resistance phenotype has combined resistance to three key antimicrobial groups: fluoroquinolones, third-generation cephalosporins and aminoglycosides [2].

Between 1-2.5% of hospitalized patients have nosocomial urinary infections, the incidence of which is variable according to the characteristics of the service in which the patients are hospitalized and of the hospital. [13] Results of various European national prevalence studies show that they account for 23 - 49% of all nosocomial infections [11].

UTIs associated with catheter use account for 30 - 40% of all nosocomial infections and are the most common source of Gram-negative bacteraemia in hospitalized patients [24].

In our study, 88.23% of *Enterobacter* strains tested were susceptible to imipenem and all at vancomicyn, while over two-thirds were resistant to ceftazidime (63.63%), ampicillin-clavulanic acid (72.41%), ciprofloxacin (76.31%), ceftriaxone (83.78%), cefepime (69.66%). Over 80% of the tested strains showed resistance to ciprofloxacin and doxycycline, almost 94% to tetracycline. The two strains tested with oxacillin were resistant. The most effective antibiotic was linezolid (in over 95%

of cases), followed by ciprofloxacin (86.36%), vancomicyn, doxycycline, and teicoplanin (over 80%).

Resistance to ampicillin-clavulanic acid was observed in 90% from isolates of *Proteus spp*., while 79.16% were resistant to cefazolin, almost 71% to ciprofloxacin and 65.21% la cefuroxime. Only half of the *Proteus* strains were susceptible to ceftriaxone.

All tested strains of *Pseudomonas aeruginosa* (figure 2) were resistant to ampicillin-clavulanic acid, ceftriaxone, cefuroxime, cefazolin, 87.5% to ceftazidime, 78.5% to ciprofloxacin. Instead, all tested strains were susceptible to vancomicyn. 17 strains from the 20 identified, were resistant to clindamycin, clarithromycin and penicillin, while 75% (15) were resistant to erytromycin and tetracycline. 55% from the isolates were resistant to piperacillin-tazobactam, consistent to ECDC analysis [2].

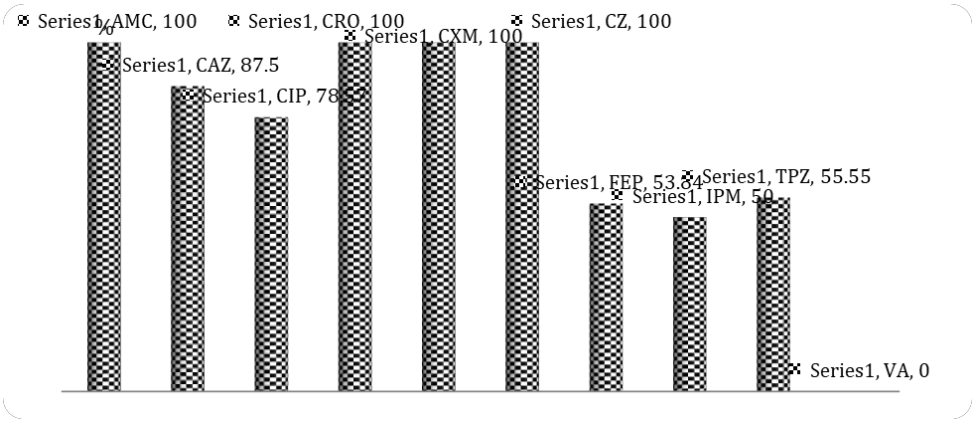

Figure 2

Resistance pattern of *Pseudomonas aeruginosa* to the tested antibiotics. AMC- ampicillin-clavulanic acid; CAZ- ceftazidime; CIP – ciprofloxacin; CRO - ceftriaxone; CXM- cefuroxime; CZ – cefazolin; FEP- cefepime; IPM - imipenem; TPZ - piperacillin-tazobactam; VA- vancomicyn

The most active antibiotic against MRSA (figure 3) was linezolid, with almost all strains (19) being sensitive. A very high level of resistance was highlighted in the case of clindamycin and clarithromycin (85%), penicillin and erytromycin (75%) and ciprofloxacin (66.66%). MRSA has been the most important cause of antimicrobial-resistant healthcare-associated infections worldwide [2], with higher percentages in the southern and south-eastern parts of Europe.

All tested strains of *Citrobacter* were resistant to ampicillin-clavulanic acid and two thirds of them at ceftazidime, cefuroxime and cefazolin. Half of them were sensitive to ceftriaxone and cefepime, and two thirds to imipenem and piperacillin-tazobactam.

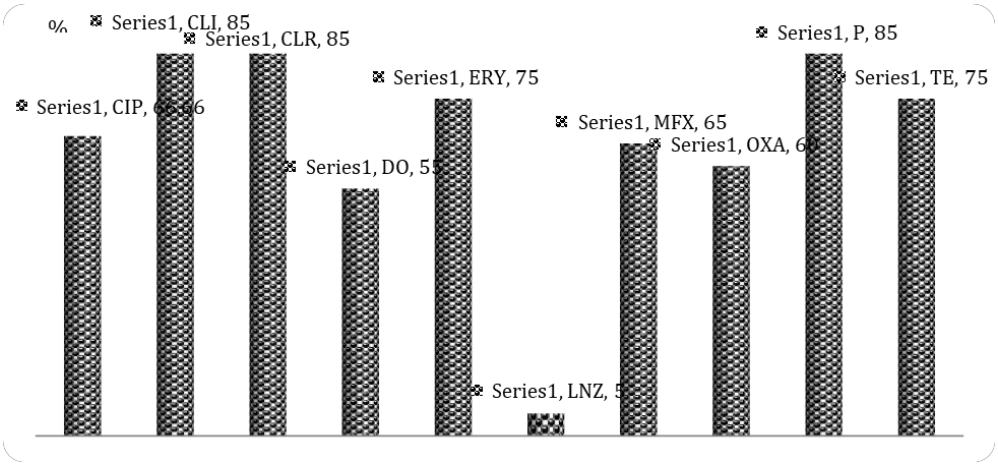

Figure 3

Resistance pattern of MRSA to the tested antibiotics. CIP – ciprofloxacin; CLI - clindamycin; CLR - clarithromycin; DO - doxycycline; ERY - erytromycin; LNZ - linezolid; MFX - moxifloxacin; OXA - oxacillin; P - penicillin; TE - teicoplanin.

The phenotypes of betalactamine resistance are presented in Table III.

Table III

Distribution of phenotypes resistant to betalactamines of the isolated bacterial strains.

Bacteria strains isolated	Wild (%)	ESBL (%)	PHN (%)	PLN (%)	CHN (%)	TRI (%)
Escherichia coli (n=205)	-	4.30	1.94	0.09	3.94	1.01
Klebsiella spp (n=94)	16.13	9.37	-	-	1.17	-
Enterobacter spp (n=37)	15.20	9.25	-	-	1.5	-
Pseudomonas aeruginosa (n=14)	-	6.58	-	-	1.27	-

ESBL: Extended-Spectrum Beta-Lactamase; PBN: Low Level Penicillinase; PHN: High Level Penicillinase; CHN: High Level Cephalosporinase; TRI: Resistant to inhibitors

Because the annual data collected by ECDC's European Antimicrobial Resistance Surveillance Network (EARS-Net) [2] consistently places Romania among the vulnerable countries, this study is the starting point of further research to highlight the evolution in time of the germ resistance.

Conclusions

The study revealed the presence of a large number of *E. coli* resistant to aminopenicillins and beta-lactamase inhibitors, of *Klebsiella, Enterobacter, Proteus*, *Pseudomonas aeruginosa* and MRSA pathogens that can induce nosocomial urinary infections.

Surveillance of antibiotic prescription and monitoring studies are required to reduce the risk of resistance, together with collaborative actions by the epidemiologist, microbiologist and clinician to adopt individual therapeutic measures, defining an antibiotic-prevention protocol or therapy, discriminating between the community and hospital strains.

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