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FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF HOSPITAL ISOLATES OF *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* IN URINE SAMPLES

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ABSTRACT

Objectives/Aim: Urinary tract infections (UTI) are a serious public health problem and caused by many pathogens, most often by *Escherichia coli* and *Klebsiella pneumoniae*. Aim of this study was to show the frequency and antimicrobial susceptibility pattern of *E. coli* and *K. pneumoniae* in hospital isolates, following the GLASS methodology.
Methods: This is a retrospective study that was conducted in the Laboratory for Urinary Infections OU Clinical Microbiology CCUS in the period from January till December 2018.
A total of 13760 urine samples were processed, using standard laboratory methods, in which significant bacteriuria was detected in 3218 (23.4%) of specimens.
Results: Out of the total number of positive samples, *E. coli* was isolated in 1166 (36.2%) and *K. pneumoniae* at 341 (10.6%) patients. The presence of *E. coli* and *K. pneumoniae* isolates is dominant in females (1103/73.2%) and age group 60 and older (812/54.7%). Out of the total *E. coli* isolates, the ESBL strain was presented with 79 (6.8%) and *K. pneumoniae* with 145 (42.5%). The proportion of carbapenemase (CPE) produced by *K. pneumoniae* isolates was 8 (2.4%). The ESBL strain distribution analysis on clinics showed the highest prevalence of both isolates at the Clinic for Nephrology: *K. pneumoniae* 26/17,9%; *E. coli*: 12/15.4%. Isolated *E. coli* showed the highest resistance to ampicillin 673/1166 (57,7%), trimethoprim-sulfamethoxazole 454/1166 (38.9%) and ciprofloxacin 253/970 (26.1%), while *K. pneumoniae* to ciprofloxacin and trimethoprim-sulfamethoxazole with 151/285 (53.0%) and 164/341 (48.1%), respectively.
Conclusions: Our study has shown that the most common cause of urinary infections in hospital settings are *E. coli* and *K. pneumoniae*. Data analysis showed that the presence of ESBL isolates was significantly higher in *K. pneumoniae* than *E. coli*. CPE isolates of *K. pneumoniae* were also detected.

Keywords: urinary tract infection, *Escherichia coli*, *Klebsiella pneumoniae*, antimicrobial resistance

INTRODUCTION

Urinary tract infections (UTI) are a serious public health problem and are caused by many pathogens, most often by *Escherichia coli* and *Klebsiella pneumoniae* [1].Uncomplicated UTI usually affects women, children, and elderly patients who are otherwise healthy. Complicated UTI is usually associated with catheter use, urinary tract abnormalities, immunosuppression or previous antibiotic therapy [2,3]. Multiple drug-resistant uropathogens are becoming more and more problematic as members of the Enterobacteriaceae family increasingly acquire expanded spectrum β-lactamase (ESBL) and produce carbapenemase (Carbapenemase-producing Enterobacteriaceae - CPE) [4].

In October 2015, the World Health Organization launched the Global Antimicrobial Resistance Surveillance System (GLASS) to support the global action plan on antimicrobial resistance. The aim is to support global surveillance and research to strengthen the evidence base on antimicrobial resistance (AMR) and help to inform decision-making and drive national, regional, and global actions [5,6].

The aim of this study was to show the incidence of urinary infections caused by *E. coli* strains and *K. pneumoniae*, distribution of infections by gender and age, the number of *E. coli* and *K. pneumoniae* ESBL strains, the proportion of *K. pneumoniae* CPE strains, distribution of ESBL strain infections

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on clinics at CCUS and antimicrobial susceptibility patterns following the GLASS methodology.

MATERIAL AND METHODS

This is a retrospective study that was conducted in the Laboratory for Urinary Infections OU Clinical Microbiology CCUS in the period from January till December 2018.

A total of 13760 urine samples were processed, of which significant bacteriuria was detected in 3218 (23.4%) of specimens. *E. coli* as a causative agent of UTI was isolated in 1166 (36,2%) and *K. pneumoniae* in 341 (10.6%) patients. The patients were men, women and children in different age groups.

Microbiological examination of urine samples was performed using standard laboratory methods including microscopic examination, cultivation to determine the number of bacteria and final identification with a biochemical series or automated systems such as Vitek2® (BioMérieux, Marcy, l'Étoile, France).

Antimicrobial susceptibility testing was performed using the standardized Kirby–Bauer disk diffusion technique, in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Antimicrobial Susceptibility Testing guidelines [7].

The following antibiotics were tested: trimethoprim-sulfamethoxazole 25 μg (TS), ciprofloxacin 5 μg (CIP), ceftriakson 30 μg (CRO), cefotaxime 30 μg (KT), ceftazidime 10 μg (CAZ), cefepim 30 μg (FEP), ampicillin 10 μg (AMP), imipenem 10 μg (IMP), meropenem 10 μg (MEM) and colistin 10 μg (COL).

RESULTS

A total of 13760 urine samples were processed, of which with significant bacteriuria there were 3218 (23.4%) samples *E. coli* as a causative agent of UTI was isolated in 1166 (36.2%) patients, *K. pneumoniae* at 341 (10.6%), while other pathogens were isolated in 1711 (53.1%) of the patients (Figure 1).

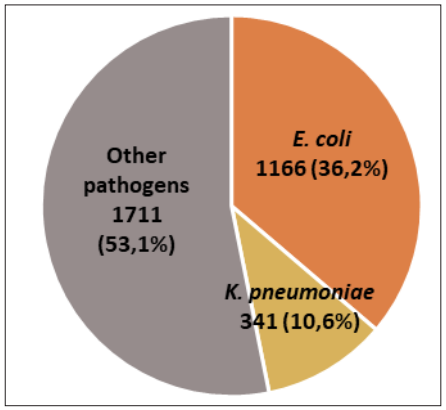

Figure 1. Incidence of urinary tract infections caused by *E. coli* and *K. pneumoniae*

Analysis of gender and age distribution showed that *E. coli* and *K. pneumoniae* isolates dominate in females with 1103 (73.2%) (Figure 2) and are most often represented in the age group 60 and older with 812 (54.7%), equally represented in the age groups of 15 - 45 and 45 - 60 years, and the least represented in the age group 5 - 15 years with 78 (5.2%) isolates (Figure 3).

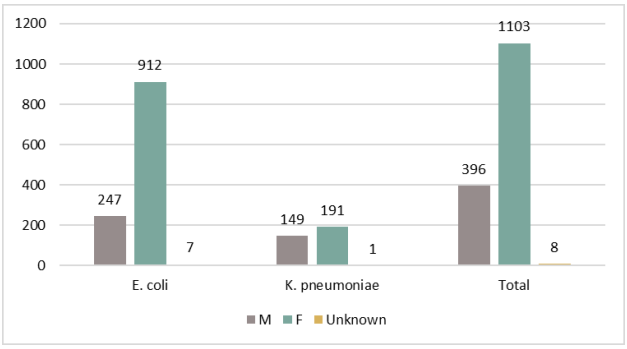

Figure 2. Incidence of urinary tract infections in relation to gender

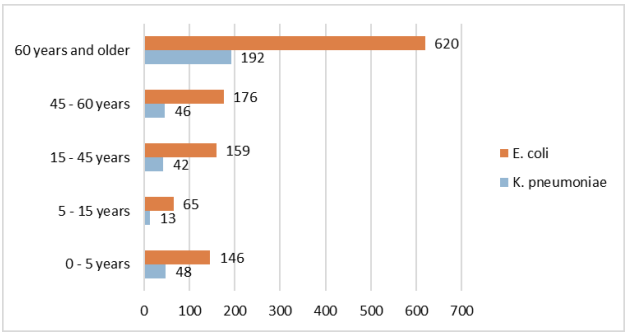

Figure 3. Incidence of urinary tract infections by age groups

Out of the total *E. coli* isolates, the ESBL strain was presented with 79 (6.8%) (Figure 4) and *K. pneumoniae* with 145 (42.5%). The proportion of carbapenemase (CPE) produced by *K. pneumoniae* isolates was 8 (2.4%) (Figure 5).

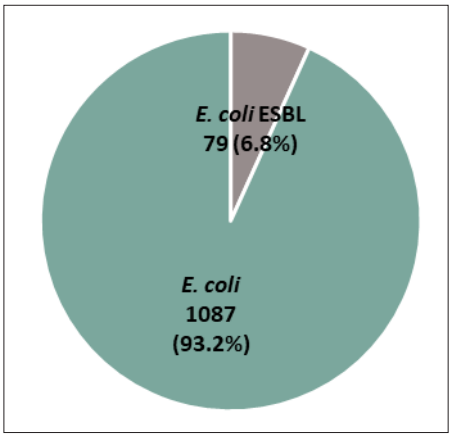

Figure 4. Distribution of *E. coli* ESBL isolates

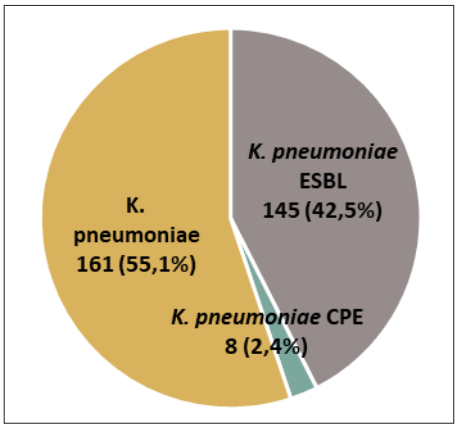

Figure 5. Distribution of *K. pneumoniae* ESBL isolates

The distribution of *E. coli* ESBL isolates in the clinics showed the highest prevalence in the Nephrology Clinic with 12 (15.2%) isolates and the Pediatric Clinic with 10 (12.7%) isolates.

Analysis of the distribution of *K. pneumoniae* ESBL isolates on clinics showed the highest prevalence in the Nephrology Clinic with 26 (17.9%) isolates and Neurological Clinic with 22 (15.2%) isolates (Figure 6).

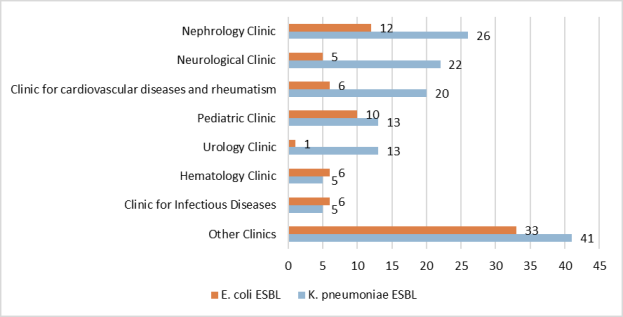

Figure 6. Distribution of *E. coli* ESBL and *K. pneumoniae* ESBL isolates on clinics at CCUS

Distribution of *K. pneumoniae* CPE showed that out of the 8 isolates, 2 isolates were at the Department for Pulmonary Diseases "Podhrastovi" while in other clinics they found one isolate (Department of Physical Medicine and Rehabilitation, Pediatric Clinic, Anesthesiology and Resuscitation Clinic, Neurological Clinic, Infectious Diseases Clinic and Psychiatric Clinic). Isolated *E. coli* showed the highest resistance to ampicillin 673/1166 (57.7%), trimethoprim-sulfamethoxazole 454/1166 (38.9%) and ciprofloxacin 253/970 (26.1%) (Table 1), while *K. pneumoniae* to ciprofloxacin and trimethoprim-sulfamethoxazole with 151/285 (53.0%) and 164/341 (48.1%) (Table 2), respectively.

Table 1. *E. coli* antibiotic susceptibility results tested according to the GLASS methodology.

E. coli				
Antibiotic	R	%	S	%
Trimethoprim-sulfamethoxazole (TS)	454	38.9	712	61.1
Ciprofloxacin (CIP)	253	26.1	717	73.9
Ceftriakson (CRO)	83	7.1	1083	92.9
Cefotaksim (KT)	10	5.1	186	94.9
Ceftazidime (CAZ)	87	7.5	1079	92.5
Cefepim (FEP)	61	70.1	26	29.9
Ampicillin (AMP)	673	57.7	493	42.3
Imipenem (IMP)	0	/	87	100.0
Meropenem (MEM)	0	/	87	100.0

Table 2. *K. pneumoniae* antibiotic susceptibility results tested according to the GLASS methodology.

K. pneumoniae				
Antibiotic	R	%	S	%
Trimethoprim-sulfamethoxazole (TS)	164	48.,%	177	51.9
Ciprofloxacin (CIP)	151	53.0	134	47.0
Ceftriakson (CRO)	158	46.3	183	53.7
Cefotaksim (KT)	12	21.4	44	78.6
Ceftazidime (CAZ)	159	46.6	182	53.4
Cefepim (FEP)	153	89.5	18	10.5
Imipenem (IMP)	8	4.7	163	95.3
Meropenem (MEM)	8	4.7	163	95.3
Colistin (COL)	0	/	8	100.0

DISCUSSION

In this study, we evaluated the incidence and distribution of urinary infections caused by *E. coli* and *K. pneumoniae,* the presence of ESBL strains and their antibiotic susceptibility pattern. A retrospective study that was conducted in the Laboratory for Urinary Infections OU Clinical Microbiology CCUS in the period from January till December 2018. Out of 13760 urine samples processed, there were 3218 (23.4%) samples with significant bacteriuria.

In our study, *E. coli* was the most frequent uropathogen with 36.2% of all isolates followed by *K. pneumoniae* with 10.6% while other pathogens were isolated in 53.1% patients. The similar frequency for isolates of *E. coli* an *K. pneumoniae* has been reported in studies from other countries such as Serbia (*E. coli*: 56.6%; *K. pneumoniae*: 16.2%), and France (*E. coli*: 41,8%, *K. pneumoniae*: 42%) [8,9].

The presence of *E. coli* and *K. pneumoniae* isolates were dominant in females (73.2%) and age group 60 and older (54.7%) and the least represented in

the age group 5 - 15 years with 5.2% isolates. Similar results were reported in studies conducted in France and Swiss [9,10]. The reasons for the high prevalence of the UTIs in females can be due to the anatomical structure of the urogenital tract having a short urethra, presence of normal flora in the vagina, menstrual cycle and pregnancy.

Out of the total *E. coli* isolates, the ESBL strain was presented with 6.8% and *K. pneumoniae* with 42.5%. Differences in distribution between ESBL isolates of *E. coli* and *K. pneumoniae* likely reflect the different characteristics of these two bacteria. *K. pneumoniae* is a bacterium that has adapted especially to the hospital environment and survives longer than other Enterobacteriaceae on hands and environmental surfaces, making it easier to cause cross-infection within hospitals [11,12].

The prevalence of bacterial isolates expressing ESBL phenotype varies across different geographical regions with low rates of 3 – 8% reported in Sweden, Japan Singapore compared to much higher prevalence rates documented in studies from Portugal (34%), Italy (37%), New York (44%), Latin American countries (30 – 60%) and Turkey (58%) [13].

The proportion of *K. pneumoniae* CPE isolates in our hospital was 2.4%. The European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE), performed in 2013–2014 in Europe, Turkey, and Israel, showed that the countries with the highest rate of carbapenem-resistant *K. pneumoniae* were Greece and Turkey, while those with the lowest were Spain and Hungary and that *K. pneumoniae* and *E. coli* produced carbapenemases, mainly KPC-type and OXA-48-like [14]. Based on our results, we have much fewer CPE isolates than in Southeast European countries.

The distribution of *E. coli* ESBL isolates in the clinics showed the highest prevalence in the Nephrology Clinic with 15.2% isolates and the Pediatric Clinic with 12.7% isolates.

Analysis of the distribution of *K. pneumoniae* ESBL isolates on clinics showed the highest prevalence in the Nephrology Clinic with 17,9% isolates and Neurological Clinic with 15.2% isolates.

Isolated *E. coli* showed the highest resistance to ampicillin (57.7%), trimethoprim-sulfamethoxazole 38,9% and ciprofloxacin (26.1%) while the most sensitivity was to imipeneme and meropeneme (100%). Similar results had the study conducted in 6 European Countries including Russia and study in Swiss where the resistance to ampicillin was 7,7 – 55,8%, to trimethoprim-sulfamethoxazole 7.7 – 41.9% and to ciprofloxacin 1.1 – 35,3% [15, 10]

In our study, *K. pneumoniae* isolates showed the highest resistance to ciprofloxacin (53,0%) and trimethoprim-sulfamethoxazole (48.1%), and due to a significant incidence of ESBL strains high resistance to cephalosporins (ceftriaxone and ceftazidime with 46.3%). *K. pneumoniae* was most sensitive to imipenem and meropenem (95,3%), while there was no resistance to colistin. A similar result was in South Korean study and Portugal where the resistance to ciprofloxacin was in a range from 41.3 – 46.5% and resistance to trimethoprim-sulfamethoxazole was 38.6 – 46.7% [16,17].

Our study has shown high resistance of *E. coli* and *K. pneumoniae* to fluoroquinolones what is similar to studies conducted in many countries [16-19].

Ciprofloxacin is the most commonly prescribed fluoroquinolone for UTIs on all levels of health care [20] and the use of this antimicrobial agent as empirical therapy for UTI should be reconsidered.

Plasmids transmitting genes encoding ESBL frequently also contain genes that encode resistance to aminoglycosides, trimethoprim-sulfamethoxazole and fluoroquinolones Although there has been only one report of plasmid-mediated quinolone resistance in *K. pneumoniae*, there is a strong association between quinolone resistance and ESBL production. The reasons for this are not fully clarified [21]. It was found that 60% of the ciprofloxacin-resistant bacteria produced and ESBL compared to 16% of bacteria that were not resistant to ciprofloxacin [21].

Prevalence of antibiotic resistance in urinary tract infections caused by *E. coli* and *K. pneumoniae* is high globally, including to some first-line treatments such as trimethoprim-sulfamethoxazole, ciprofloxacin and third-generation cephalosporins.

Substantial variations in antibiotic use exist globally, with over-the-counter availability common in many countries. Primary care clinicians should consider the impact of any antibiotic use on subsequent antimicrobial resistance and avoid their unnecessary use by following local and national guidance whenever possible.

The improved infrastructure of primary care, access to healthcare, and antibiotic regulation might be necessary to reduce the burden of antimicrobial resistance.

CONCLUSION

Resistance to antibiotics is a serious health problem when developing in a bacterial species such as *E. coli* and *K. pneumoniae*.

Antibiotic consumption in many areas exceeds real needs and unjustifiably promotes resistance development, and once-produced resistant strains are easily spread in the community, especially if the genetic material encoding resistance enters the strain with a high epidemic and virulent potential.

A better definition of epidemiologically important strains and monitoring of their reservoirs and modes of transmission would be the imperative limiting the spread of resistance and preserving the efficacy of antibiotics.

SAŽETAK

Uvod: Infekcije urinarnog trakta (IUT) su ozbiljan javno zdravstveni problem i uzrokovane su nizom patogena, a najčešće su *Escherichia coli* i *Klebsiella pneumoniae*.
Ovaj rad ima za **cilj** prikazati učestalost i antimikrobnu osjetljivost bolničkih izolata *E. coli* i *K. pneumoniae*, a u skladu sa metodologijom GLASS mreže.
Metode: Ovo je retrospektivna studija koja je sprovedena u laboratoriju za urinarne infekcije OJ Klinička mikrobiologija KCUS u periodu od 01.01. do 31.12.2018. godine.
Ukupno je obrađeno 13760 uzoraka urina, standardnim laboratorijskim metodama, kod kojih je signifikantna bakteriurija dokazana u 3218 (23.4%) uzoraka.
Rezultati: Od ukupnog broja pozitivnih uzoraka *E. coli* je izolirana kod 1166 (36.2%), a *K. pneumoniae* kod 341 (10.6%) pacijenata. Zastupljenost izolata *E. coli* i *K. pneumoniae* dominantna je u ženskom spolu (1103/73.2%) i u dobnoj skupini 60 i više godina (812/ 54.7%). Od ukupnog broja izolata *E. coli* udio ESBL sojeva je bio 79 (6.8%), a kod *K. pneumoniae* iznosio je 145 (42.5%). Udio izolata *K. pneumoniae* koji produkuju karbapenemaze (CPE) je iznosio 8 (2,4%). Analiza distribucije ESBL sojeva po klinikama pokazala je najveću zastupljenost oba izolata na Klinici za nefrologiju: *K. pneumoniae* 26/17.9%); *E. coli*: 12/15.4%. Izolati *E. coli* su pokazali najveću rezistenciju na ampicilin 673/1166 (57.7%), trimethoprim-sulfamethoxazole 454/1166 (38.9%) i ciprofloksacin 253/970 (26.1%), dok su izolati *K. pneumoniae* pokazali najveću rezistenciju na ciprofloksacin i trimethoprim-sulfamethoxazole 151/285 (53.0%) i 164/341 (48.1%).
Zaključak: Naša je studija pokazala da su najčešći uzročnici urinarnih infekcija u bolničkoj sredini *E. coli* i *K. pneumoniae*. Analiza podataka je pokazala da je zastupljenost ESBL izolata značajno veća kod *K. pneumoniae* u odnosu na *E. coli*. Dokazana je i pojava CPE izolata *K. pneumoniae*.

Ključne riječi: infekcije urinarnog trakta, *Escherichia coli*, *Klebsiella pneumoniae*, antimikrobna rezistencija

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