International Journal of Biomedicine 13(2) (2023) 313-320
http://dx.doi.org/10.21103/Article13(2)_OA22

ORIGINAL ARTICLE

Antimicrobial Resistance

INTERNATIONAL JOURNAL OF BIOMEDICINE

Prevalence of Isolated Bacteria from Urinary Tracts and Antibiotic Resistance Trend in Peja Region, Kosovo

Ilirjana Loxhaj[1,2], Sanije Hoxha-Gashi[3,4*], Sunchica Petrovska[1], Sadushe Loxha[3]

[1]*Faculty of Medicine, University "Ss.Cyril and Methodius", Skopje, North Macedonia*
[2]*Regional Hospital of Peja, Kosovo*
[3]*Faculty of Medicine, University of Pristina "Hasan Prishtina", Pristina, Kosovo*
[4]*National Institute of Public Health of Kosovo, Pristina, Kosovo*

Abstract

Background: Urinary tract infections are the most frequent bacterial infections, causing significant morbidity at a high cost of effectiveness. The main purpose of the research was to determine the prevalence and the resistance of gram-negative bacteria in urine samples in the Peja region.

Methods and Results: This cohort longitudinal, prospective-retrospective study was conducted in the microbiological laboratories of the regional hospital in Peja and the Regional Centre of Public Health in Peja. The research includes all urine samples tested for gram-negative bacteria from 2018 to 2020. A total of 12,791 urine samples were analyzed in the study, of which 2316 (18.11%) were positive for the growth of gram-negative pathogenic strains, and 10,479 (81.89%) were negative. The most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp., *Klebsiella* spp., *Acinetobacter* spp., and *Pseudomonas aeruginosa* (5.18%, 4.79%, 2.42% and 2.37% respectively). From the data of our research, we can conclude that *E. coli*, *Proteus* spp., and *Klebsiella* spp. were the three commonly isolated microorganisms in the Peja region.

A trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020; to tobramycin - from 3.68% in 2018 to 5.97% in 2020; to cefalexin from 29.41% in 2018 to 31.09% in 2020; to cefuroxime from 23.7% in 2018 to 28.99% in 2020; to cefotaxime from 21.32% in 2018 to 27.94% in 2020; ceftazidime from 18.84% in 2018 to 27.54% in 2020; to piperacillin from 28.73% in 2018 to 34.97% in 2020; to nitrofurantoin from 5.98% in 2018 to 8.21% in 2020; and to trimethoprim/sulfamethoxazole from 35.56% in 2018 to 42.77% in 2020. In the analyzed period, a trend of the increased resistance of *Proteus* spp. to ampicillin was registered from 31.43% in 2018 to 81.25% in 2020 and to imipenem from 4.76% in 2018 to 12% in 2020. The resistance rates of *Klebsiella* spp. strains isolated in 2020 (100% to ampicillin, 5% to amikacin, 38.46% to ofloxacin, 8.7% to imipenem, 33.33% to nitrofurantoin) were higher than those reported in 2018 (87.5%, 2.94%, 34.62%, 6.25%, and 28.21%, respectively).

Conclusion: Data from this study can be used to control antibiotic susceptibility trends, create local antibiotic policies, and help clinicians in the rational choice of antibiotic therapy, thereby preventing indiscriminate antibiotic use.(**International Journal of Biomedicine. 2023;13(2):313-320.**)

Keywords: urinary tract infections • gram-negative bacteria • antibiotic resistance

For citation: Loxhaj I, Hoxha-Gashi S, Petrovska S, Loxha S. Prevalence of Isolated Bacteria from Urinary Tracts and Antibiotic Resistance Trend in Peja Region, Kosovo. International Journal of Biomedicine. 2023;13(2):313-320. doi:10.21103/Article13(2)_OA22

Introduction

Urinary tract infections (UTIs) are the most frequent bacterial infections, causing significant morbidity at a high cost of effectiveness. They are one of the most prevalent infections in both the community (accounting for 10%-30% of infections in primary care) and hospital settings (30-40%).[1-3] The presence of bacteria in urine more than 10^5/ml causes UTIs.[4] The pathogens that cause UTIs are different. The most common bacterial pathogens isolated from infected patients' urinary tracts are *E. coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, and Enterococcus species.[5-7] Uropathogenic *E. coli* (UPEC) is the dominant infectious agent in both uncomplicated and complicated UTIs. *Enterococcus* spp. and *Candida* spp. are substantially more common in complicated infections, while *Staphylococcus saprophyticus* is rare.[8] Infection with UPEC increases the likelihood of recurrence within 6 months.[9,10]

UTIs are a problem that affects all age groups and genders, but the most predisposed are women, given the anatomical construction of the urethra and the greater possibility of contamination with bacterial flora of the region. Slowing urine output at older ages makes these ages more vulnerable; greater aggressiveness of bacterial virulence in the urinary tract also increases the chance of UTIs.[11,12] MDR infections worldwide cause 700,000 deaths, which number could increase to 10 million by 2050, depending on the discovery of new antibiotics and the type of resistance.[13,14] Estimated costs are 3.8% of GDP.[15] Infections caused by gram-negative bacteria resistant to antibiotics in recent years around the world and in our country have increased enormously and are endangering the lives of patients by rendering them unable to be treated. Given that for the treatment of these infections caused by pathogens resistant to more antibiotics, clinicians do not have many choices for an effective antimicrobial; then it remains important to control and monitor the spread of resistance.[16,17] Resistance of gram-negative bacteria to antibiotics is increasing in Europe, where of particular importance is the increase of antibiotic resistance of *E. coli*, which have shown an increasing trend of antimicrobial resistance to first-line antibiotics, ciprofloxacin and trimethoprim/sulfamethoxazole. Some studies have shown an increase in the resistance of *E. coli* in UTIs for these antibiotics to 20%-45% of the isolates.[18,19] Antibiotic use in the last 7 years in the EU decreased by 6%, but there are differences between different countries. Denmark, Latvia, the Netherlands, and Romania reduced consumption by up to 9%, while Italy and Spain increased it by 9%.[20] According to a study conducted in Kosovo in 2017, more than half of respondents (58.7%) have used antibiotics during the past year, while in the EU for 2013 - 35%. The results were higher than southern European countries such as Malta (48.0%), Cyprus (47.0%), and Romania (47.0%) while much higher than Sweden (24.0%), Poland (26.0%), and Germany (27.0%).[21]

The main purpose of the research was to determine the prevalence and the resistance of gram-negative bacteria in urine samples in the Peja region.

Materials and Methods

This cohort longitudinal, prospective-retrospective study was conducted in the microbiological laboratories of the regional hospital in Peja and the Regional Centre of Public Health in Peja.

The research includes all urine samples tested for gram-negative bacteria from 2018 to 2020. The epidemiological method was used, more precisely its descriptive part, to identify the results obtained from the database. The presence of gram-negative bacteria and their resistance to antibiotics was analyzed. The survey includes both male and female respondents over the age of 18. The methodology used was both quantitative and qualitative. All pathogenic isolates were studied, with a focus on gram-negative pathogenic bacteria and their medication resistance. Exclusion criteria were isolates from patients under the age of 18; patients with more than two species of bacteria, and isolates of *Candida* spp.[22]

Procedures

Bacteriological examination of urine samples

Microbiological laboratory examination of urine samples, urine culture (UC) is performed in the microbiological laboratories based on the standards set by EUCAST. The urine specimen for UC is usually preferred to be the morning specimen or with the patient not urinating for 4 hours prior to sampling. Significant bacteriuria is considered according to the criteria formulated by many authors, the presence of many bacteria of 100,000 and more per ml (10^5 CFU/mL of urine) with one or two pathogenic types of bacteria in culture. Then we identify the bacteria and test sensitivity to antibiotics. If both types of bacteria are non-pathogenic in the culture, then we are dealing with contamination of the sample and in this case, we only identify the bacteria and do not test sensitivity to antibiotics. A urine sample for UC is taken by spontaneous micturition. Before sampling, it is preferable: to clean the genital area to take from the middle stream of urine, not more than 2ml. in sterile plastic bottles. The time from taking to processing the sample should be 30 min to 2 hours; otherwise, the sample should be stored in the refrigerator at a temperature from +2°C to +8°C. Cultivation is done in nutrient media for urine (Blood Agar, MacConkey agar); inoculum is in the amount of 0.001 mL. The culture is incubated at 37°C for 18-24h. On the second day after incubation of the urine sample, the culture suspected of gram-negative bacteria is processed to identify these bacteria. automatic equipment (VITEK 2) is lacking, gram-negative bacteria can be identified by testing biochemical properties through a short biochemical series that consists of 1)Breakdown of carbohydrates (Kligler Iron Agar); 2)Breakdown of tryptophan (indole); 3)Decomposition of urea in deep agar; 4)Mobility test; 5)Using citrates (Simons Citrate Agar). Incubation is done aerobically at 37°C for another 18-24 hours. On the second day, in addition to processing the urine sample for identification of bacteria, antibiotic susceptibility testing is performed.[23]

Determination of susceptibility to antibiotics

Susceptibility testing of gram-negative bacteria to antibiotics in Kosovo is done based on the standards set by EUCAST, with the method of disk diffusion in plates in the nutrient medium. From the culture grown after 24h incubation on MacConkey agar, the procedure for preparing the antibiogram is as follows:

- With sterile loops, 2-3 colonies of bacteria are taken from the medium, then placed in a test tube containing physiological saline (3 mL)
- The turbidity of the test tube is compared with the standard until turbidity is reached (0.5 McFarland)
- Sterile swabs are inserted into the suspension test tube and then removed by removing excess fluid through the walls of the test tube
- The bacteria are sown on the agar surface in three directions
- Under aseptic conditions, antibiotic discs are placed with sterile forceps at 30 mm from each other.

The media planted in this way are incubated at 37°C for 18-24 h. During this time, the antibiotic diffuses from the disk in the agar. The bacteria will grow around the disks depending on their sensitivity to the antibiotic. The result is assessed by measuring the area of inhibition around which there is no growth of bacterial colonies. The categories of sensitivity are S-sensitive, I-intermediary, and R-resistant. Antibiotics used for antibiotic susceptibility testing of urine samples in the microbiological laboratories were ampicillin (10 µg), cefalexin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), gentamicin (30 µg), tobramycin (10 µg), amikacin (30 µg), ofloxacin (10 µg), trimethoprim-sulfamethoxazole (25 µg), nitrofurantoin (100 µg), piperacillin (30 µg), imipenem (10 µg). Microbiological analysis of urine takes 48h until final identification of bacteria and antibiotic susceptibility testing.

Data were collected from the database of Peja Hospital and Regional Center of Public Health in Peja, while for access to the data, permission was obtained from the managers, who were informed about the purpose of the research, positive aspects, and benefits of the study results.

Statistical analysis was performed using statistical software package SPSS version 22.0 (SPSS Inc, Armonk, NY: IBM Corp). The frequencies of categorical variables were compared using a chi-squared test, and a compare proportions test was applied. A probability value of $P<0.05$ was considered statistically significant.

Results

A total of 12,791 urine samples were analyzed in the study, of which 2316(18.11%) were positive for the growth of gram-negative pathogenic strains, and 10,479(81.89%) were negative. Table 1 shows the distribution of positive and negative urine cultures in total for the period 2018-2020, as well as for each year separately.

Table 1.

Distribution of positive and negative urine cultures for the growth of gram-negative pathogenic strains by years.

Year	Urine cultures		
	Positive n (%)	Negative n (%)	Total
2018	878 (17.82)	4050 (82.18)	4928
2019	977 (18.8)	4218 (81.19)	5195
2020	461 (17.28)	2207 (82.72)	2668
Total	2316 (18.11)	10475 (81.89)	12791

From a total of 2316 gram-negative isolates, the most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp. (5.18%), *Klebsiella* spp. (4.79%), *Acinetobacter* spp. (2.42%), and *Pseudomonas aeruginosa*, the last isolated in a very small percentage of samples (2.37%). By years: the prevalence of *E. coli* was 83.49%, 83.83%, and 81.34%, respectively, in 2018, 2019, and 2020, without a statistically significant difference P=0.48); the prevalence of *Klebsiella* spp. was 4.78%, 4.71%, and 4.99%, respectively, in 2018, 2019 and 2020, without a statistically significant difference (P=0.97); the prevalence of *Proteus* spp. was 4.1%, 5.22%, and 7.16%, respectively, in 2018, 2019 and 2020, without a statistically significant difference (P=0.056); the prevalence of *Pseudomonas aeruginosa* was 2.39%, 2.46%, and 2.17%, respectively, in 2018, 2019 and 2020, without a statistically significant difference (P=0.94); the prevalence of *Acinetobacter* spp. was 3.42, 1.84% and 1.74%, respectively, in 2018, 2019 and 2020, with a statistically significant difference in prevalence between 2018 and 2019 (P=0.0324) (Table 2).

Table 2.

Prevalence of gram-negative bacteria by years

Bacteria	Total	2018	2019	2020	*P*-value
	N=2316 (%)	n=878 (%)	n=977 (%)	n=461 (%)	
E. coli	1927 (83.2)	733 (83.49)	819 (83.33)	375 (81.34)	0.48
Klebsiella spp.	111 (4.79)	42 (4.78)	46 (4.71)	23 (4.99)	0.97
Proteus spp.	120 (5.18)	36 (4.10)	51 (5.22)	33 (7.16)	0.056
P. aeruginosa	55 (2.37)	21 (2.39)	24 (2.46)	10 (2.17)	0.94
Acinetobacter spp.	56 (2.42)	30 (3.42)	18 (1.84)	8 (1.74)	0.050 0.0324*
Others	47 (2.03)	16 (1.82)	19 (1.94)	12 (2.60)	0.610

**- 2018 vs. 2019*

In the analyzed period, a trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020; to tobramycin - from 3.68% in 2018 to 5.97% in 2020; to cefalexin from 29.41% in 2018 to 31.09% in 2020; to cefuroxime from 23.7% in 2018 to 28.99% in 2020; to cefotaxime from 21.32% in 2018 to 27.94% in 2020; ceftazidime from 18.84% in 2018 to 27.54% in 2020; to piperacillin from 28.73% in 2018 to 34.97% in 2020; to nitrofurantoin from 5.98% in 2018 to 8.21% in 2020; and to trimethoprim/sulfamethoxazole from 35.56% in 2018 to 42.77% in 2020.

Increased resistance of *E. coli* to ampicillin in 2019 versus 2018, and in 2020 vs. 2018 and 2019 was statistically significant ($P<0.0001$), as well as to cefalexin in 2020 vs. 2019 (P=0.0314), to nitrofurantoin in 2019 vs. 2018 (P=0.017), and to trimethoprim/sulfamethoxazole in 2020 vs. 2018 (P=0.0247) (Table 3).

The resistance rates of *Klebsiella* spp. strains isolated in 2020 (100% to ampicillin, 5% to amikacin, 38.46% to ofloxacin, 8.7% to imipenem, 33.33% to nitrofurantoin) were higher than those reported in 2018 (87.5%, 2.94%, 34.62%, 6.25%, and 28.21%, respectively). The difference in the resistance of *Klebsiella* spp. to ampicillin in 2020 vs. 2019 was statistically significant (P=0.0075) (Table 4).

Table 3.
Distribution of resistant E. coli strain isolates by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	92	35/40 (87.5)	34/46 (73.91)	23/23 (100)	P_{1-2}=0.1166; P_{1-3}=0.0796; P_{2-3}=0.0075
Amikacin	2	1/34 (2.94)	0/45 (0)	1/20 (5)	P_{1-2}=0.2501; P_{1-3}=0.7013; P_{2-3}=0.1336
Gentamicin	16	7/41 (17.07)	6/45 (13.33)	3/23 (13.04)	P_{1-2}=0.6306; P_{1-3}=0.6725; P_{2-3}=0.9736
Tobramycin	7	4/25 (16)	3/23 (13.04)	0/14 (0)	P_{1-2}=0.7739; P_{1-3}=0.1189; P_{2-3}=0.1644
Cefalexin	47	14/26 (53.85)	22/43 (51.16)	11/21 (52.38)	P_{1-2}=0.8296; P_{1-3}=0.9209; P_{2-3}=0.9275
Cefuroxime	30	12/25 (48)	12/24 (50)	6/14 (42.86)	P_{1-2}=0.8898; P_{1-3}=0.7605; P_{2-3}=0.6748
Cefotaxime	29	12/26 (46.15)	11/24 (45.83)	6/14 (42.86)	P_{1-2}=0.9821; P_{1-3}=0.8438; P_{2-3}=0.8609
Ceftazidime	26	12/26 (46.15)	8/15 (53.33)	6/14 (42.86)	P_{1-2}=0.6617; P_{1-3}=0.8438; P_{2-3}=0.5796
Ofloxacin	17	9/26 (34.62)	3/12 (25)	5/13 (38.46)	P_{1-2}=0.5584; P_{1-3}=0.8161; P_{2-3}=0.4800
Imipenem	8	2/32 (6.25)	4/44 (9.09)	2/23 (8.7)	P_{1-2}=0.6525; P_{1-3}=0.7324; P_{2-3}=0.9580
Piperacillin	55	24/36 (66.67)	18/40 (45)	13/22 (59.09)	P_{1-2}=0.0595; P_{1-3}=0.5634; P_{2-3}=0.2923
Nitrofurantoin	30	11/39 (28.21)	13/37 (35.14)	6/18 (33.33)	P_{1-2}=0.5187; P_{1-3}=0.6971; P_{2-3}=0.8956
Trimethoprim /Sulfamethoxazole	57	21/36 (58.33)	23/39 (58.97)	13/22 (59.09)	P_{1-2}=0.9555; P_{1-3}=0.9549; P_{2-3}=0.9928

R-resistance; n-number of patients

Table 4.
Distribution of resistant isolates of Klebsiella spp. by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	92	35/40 (87.5)	34/46 (73.91)	23/23 (100)	P_{1-2}=0.1166; P_{1-3}=0.0796; P_{2-3}=0.0075
Amikacin	2	1/34 (2.94)	0/45 (0)	1/20 (5)	P_{1-2}=0.2501; P_{1-3}=0.7013; P_{2-3}=0.1336
Gentamicin	16	7/41 (17.07)	6/45 (13.33)	3/23 (13.04)	P_{1-2}=0.6306; P_{1-3}=0.6725; P_{2-3}=0.9736
Tobramycin	7	4/25 (16)	3/23 (13.04)	0/14 (0)	P_{1-2}=0.7739; P_{1-3}=0.1189; P_{2-3}=0.1644
Cefalexin	47	14/26 (53.85)	22/43 (51.16)	11/21 (52.38)	P_{1-2}=0.8296; P_{1-3}=0.9209; P_{2-3}=0.9275
Cefuroxime	30	12/25 (48)	12/24 (50)	6/14 (42.86)	P_{1-2}=0.8898; P_{1-3}=0.7605; P_{2-3}=0.6748
Cefotaxime	29	12/26 (46.15)	11/24 (45.83)	6/14 (42.86)	P_{1-2}=0.9821; P_{1-3}=0.8438; P_{2-3}=0.8609
Ceftazidime	26	12/26 (46.15)	8/15 (53.33)	6/14 (42.86)	P_{1-2}=0.6617; P_{1-3}=0.8438; P_{2-3}=0.5796
Ofloxacin	17	9/26 (34.62)	3/12 (25)	5/13 (38.46)	P_{1-2}=0.5584; P_{1-3}=0.8161; P_{2-3}=0.4800
Imipenem	8	2/32 (6.25)	4/44 (9.09)	2/23 (8.7)	P_{1-2}=0.6525; P_{1-3}=0.7324; P_{2-3}=0.9580
Piperacillin	55	24/36 (66.67)	18/40 (45)	13/22 (59.09)	P_{1-2}=0.0595; P_{1-3}=0.5634; P_{2-3}=0.2923
Nitrofurantoin	30	11/39 (28.21)	13/37 (35.14)	6/18 (33.33)	P_{1-2}=0.5187; P_{1-3}=0.6971; P_{2-3}=0.8956
Trimethoprim /Sulfamethoxazole	57	21/36 (58.33)	23/39 (58.97)	13/22 (59.09)	P_{1-2}=0.9555; P_{1-3}=0.9549; P_{2-3}=0.9928

R-resistance; n-number of patients

In the analyzed period, a trend of the increased resistance of *Proteus* spp. to ampicillin was registered from 31.43% in 2018 to 81.25% in 2020 and to imipenem from 4.76% in 2018 to 12% in 2020. The increased resistance of *Proteus* spp. to ampicillin in 2020 vs. 2018 and 2020 vs. 2019 was statistically significant (P<0.0001, and P=0.0037, respectively). In addition, we found the increased resistance of *Proteus* spp. to imipenem in 2020 vs. 2019 (P=0.0211) (Table 5).

Table 5.
Distribution of resistant isolates of Proteus spp. by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	61	11/35 (31.43)	24/49 (48.98)	26/32 (81.25)	P_{1-2}=0.1098; P_{1-3}<0.0001; P_{2-3}=0.0037
Amikacin	2	0/18 (0)	1/47 (2.13)	1/27 (3.7)	P_{1-2}=0.5358; P_{1-3}=0.4145; P_{2-3}=0.6905
Gentamicin	18	4/35 (11.43)	9/50 (18)	5/31 (16.13)	P_{1-2}=0.4103; P_{1-3}=0.5816; P_{2-3}=0.8298
Tobramycin	4	1/8 (12.5)	3/10 (30)	0/6 (0)	P_{1-2}=0.3885; P_{1-3}=0.3865; P_{2-3}=0.1495
Cefalexin	20	0/7 (0)	13/48 (27.08)	7/26 (26.92)	P_{1-2}=0.1185; P_{1-3}=0.1278; P_{2-3}=0.9883
Cefuroxime	5	2/9 (22.22)	3/10 (30)	0/7 (0)	P_{1-2}=0.7082; P_{1-3}=0.1967; P_{2-3}=0.1213
Cefotaxime	3	1/9 (11.11)	2/10 (20)	0/6 (0)	P_{1-2}=0.6055; P_{1-3}=0.4142; P_{2-3}=0.2568
Ceftazidime	3	1/9 (11.11)	2/7 (28.57)	0/7 (0)	P_{1-2}=0.3901; P_{1-3}=0.3778; P_{2-3}=0.1410
Ofloxacin	2	0/9 (0)	1/7 (14.29)	1/7 (14.29)	P_{1-2}=0.2568; P_{1-3}=0.2568; P_{2-3}=1.0000
Imipenem	4	1/21 (4.76)	0/43 (0)	3/25 (12)	P_{1-2}=0.1525; P_{1-3}=0.3906; P_{2-3}=0.0211
Piperacillin	16	5/22 (22.73)	6/31 (19.35)	5/22 (22.73)	P_{1-2}=0.7671; P_{1-3}=1.0000; P_{2-3}=0.7671
Nitrofurantoin	60	21/34 (61.76)	26/44 (59.9)	13/24 (54.17)	P_{1-2}=0.8684; P_{1-3}=0.5666; P_{2-3}=0.6499
Trimethoprim /Sulfamethoxazole	50	16/35 (45.71)	22/47 (46.81)	12/28 (42.86)	P_{1-2}=0.9218; P_{1-3}=0.8224; P_{2-3}=0.7413

R-resistance; n-number of patients

Table 6.
Distribution of resistant isolates of Pseudomonas aeruginosa by years.

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	41	15/19 (78.95)	17/18 (94.44)	9/9 (100)	P_{1-2}=0.1742; P_{1-3}=0.1443; P_{2-3}=0.4793
Amikacin	6	1/12 (8.33)	4/24 (16.67)	1/9 (11.11)	P_{1-2}=0.5012; P_{1-3}=0.8340; P_{2-3}=0.6961
Gentamicin	12	3/21 (14.29)	7/23 (30.43)	2/10 (20)	P_{1-2}=0.2071; P_{1-3}=0.6910; P_{2-3}=0.5426
Tobramycin	1	0/4 (0)	1/8 (12.5)	0/2 (0)	P_{1-2}=0.4795; P_{2-3}=0.6171
Cefalexin	22	1/1 (100)	14/17 (82.35)	7/9 (77.78)	P_{1-2}=0.6547; P_{1-3}=0.6171; P_{2-3}=0.7827
Cefuroxime	7	3/4 (75)	4/4 (100)	0/1 (0)	P_{1-2}=0.3173; P_{1-3}=0.2207; P_{2-3}=0.0455
Cefotaxime	7	3/4 (75)	4/5 (80)	0/1 (0)	P_{1-2}=0.8658; P_{1-3}=0.2207; P_{2-3}=0.1573
Ceftazidime	9	1/4 (25)	7/9 (77.78)	1/2 (50)	P_{1-2}=0.0828; P_{1-3}=0.5762; P_{2-3}=0.4468
Ofloxacin	2	1/3 (33.33)	1/3 (33.33)	0/1 (0)	P_{1-2}=1.0000; P_{1-3}=0.5637; P_{2-3}=0.5637
Imipenem	8	2/15 (13.33)	4/24 (16.67)	2/7 (28.57)	P_{1-2}=0.7813; P_{1-3}=0.3990; P_{2-3}=0.4903
Piperacillin	20	6/17 (35.29)	10/16 (62.5)	4/9 (44.44)	P_{1-2}=0.1237; P_{1-3}=0.6546; P_{2-3}=0.3922
Nitrofurantoin	37	17/20 (85)	15/23 (65.22)	5/6 (83.33)	P_{1-2}=0.1428; P_{1-3}=0.9223; P_{2-3}=0.4014
Trimethoprim /Sulfamethoxazole	39	16/20 (80)	18/20 (90)	5/8 (62.5)	P_{1-2}=0.3819; P_{1-3}=0.3428; P_{2-3}=0.0919

R-resistance; n-number of patients

The resistance rates of *P. aeruginosa* strains isolated in 2020 (100% to ampicillin and 28.57% to imipenem) were higher than those reported in 2018 (78.95% and 13.33%, respectively). The difference in the reduced rate of resistance of *P. aeruginosa* to cefuroxime in 2020 vs. 2019 (*P*=0.0455) was statistically significant (Table 6).

Table 7.
Distribution of resistant isolates of Acinetobacter spp. by years

Antibiotic	Year				Statistics
	Total	2018 R/n (%) [1]	2019 R/n (%) [2]	2020 R/n (%) [3]	
Ampicillin	38	16/30 (53.33)	16/18 (88.89)	6/8 (75)	P_{1-2}=0.0123; P_{1-3}=0.2764; P_{2-3}=0.3743
Amikacin	6	1/14 (7.14)	4/18 (22.22)	1/7 (14.29)	P_{1-2}=0.2513; P_{1-3}=0.6076; P_{2-3}=0.6628
Gentamicin	21	12/30 (40)	8/17 (47.06)	1/7 (14.29)	P_{1-2}=0.6417; P_{1-3}=0.2057; P_{2-3}=0.1401
Tobramycin	No resistance				
Cefalexin	15	0/2 (0)	11/17 (64.71)	4/7 (57.14)	P_{1-2}=0.0879; P_{1-3}=0.1763; P_{2-3}=0.7332
Cefuroxime	1	0/2 (0)	0/0	1/1 (100)	
Cefotaxime	1	0/2 (0)	0/0	1/1 (100)	
Ceftazidime	1	0/2	0/0	1/1 (100)	
Ofloxacin	2	1/2 (50)	0/0	1/1 (100)	
Imipenem	1	0/17 (0)	1/14 (7.14)	0/7 (0)	
Piperacillin	15	6/20 (30)	5/8 (62.5)	4/7 (57.14)	P_{1-2}=0.1183; P_{1-3}=0.2092; P_{2-3}=0.8382
Nitrofurantoin	30	14/26 (53.85)	14/17 (82.35)	2/5 (40)	P_{1-2}=0.0581; P_{1-3}=0.5766; P_{2-3}=0.0678
Trimethoprim /Sulfamethoxazole	32	17/26 (65.38)	11/17 (64.71)	4/8 (50)	P_{1-2}=0.9645; P_{1-3}=0.4406; P_{2-3}=0.4926

R-resistance; n-number of patients

Table 8.
Distribution of isolated gram-negative bacteria (%) in relation to number of antibiotics

Bacteria	Number of antibiotics to which resistance exists										
	0	1	2	3	4	5	6	7	8	9	10
E. coli (n=1927)	39.44	23.35	16.81	9.65	4.57	2.13	0.93	1.35	1.4	0.31	0.05
Klebsiella spp. (n=111)	9.91	18.02	13.51	16.22	9.01	4.5	5.41	6.3	9.91	5.41	1.8
Proteus spp. (n=120)	15	28.33	23.33	15.83	10	4.17	1.67	0.83	0.83	0	0
P. aeruginosa (n=55)	3.64	9.09	10.91	20	25.45	12.73	7.27	5.45	5.45	0	0
Acinetobacter spp. (n=56)	14.29	19.64	5.36	12.5	26.79	17.86	1.79	0	1.79	0	0

No trend of the increased resistance of *Acinetobacter* spp. to any of the tested antibiotics was registered. The difference in the reduced resistance rate of *P. aeruginosa* to cefalexin in 2019 vs. 2018 (*P*=0.034) and 2020 vs. 2018 (*P*=0.02) was statistically significant.

The resistance rates of *Acinetobacter* spp. strains isolated in 2020 (75% to ampicillin, 14.29% to amikacin, 57.14% to cefalexin, and 57.14% to piperacillin) were higher than those reported in 2018 (53.33%, 7.14%, 0% and 30.0%, respectively). The difference in the increased rate of resistance of *Acinetobacter* spp. to ampicillin in 2019 vs. 2018 was statistically significant (*P*=0.0123) (Table 7).

Table 8 shows the distribution of isolated gram-negative bacteria in relation to the number of antibiotics to which they show resistance. Resistance to more than one antibiotic was presented by 37.21% of bacteria from the strain *E. coli*, 72.07% from the strain *Klebsiella* spp., 56.67% from the strain *Proteus* spp., 87.27% from the strain *P. aeruginosa*, and 66.07% from the strain *Acinetobacter* spp.

Discussion

Current research examines the prevalence of UTIs, as well as the pathogens involved in infection and their sensitivity profile. There were 2316(18.11%) patients with significant bacteriuria among the 12,791 urinary specimens collected during this research. In Nigeria, the National Hospital Abuja had a lower incidence rate (13.1%).[24] In the Saudi Arabian research, the frequency was higher (32.6%).[12] In Peja Region, from a total of 2316 gram-negative isolates, the most frequently isolated bacteria were *E. coli* (83.2%), followed by *Proteus* spp. (5.15%), *Klebsiella* spp. *(4.79%)*, *Acinetobacter* spp. (2.42%*)*, and *P. aeruginosa* (2.37%*)*, the last isolated in a very small percentage of samples. *E. coli* was the most prevalent aetiologic agent isolated (80.5%) in a study conducted by Raka et al.[25] in Kosovo in 2001, followed by *Proteus spp.* (6.1%), *Klebsiella spp.* (5.9%), *Citrobacter* (5.1%), and *Mycobacterium tuberculosis* (0.8%). *E. co*li was also the most common bacteria in another research. During the 2013-2014 research on Tohid Hospital in Sanandaj,[26] the most common isolated pathogen was Escherichia coli, which was responsible for 63.09% of positive cultures.

By years, in Peja Region, the difference in prevalence of *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas aeruginosa* was not significant; only for *Acinetobacter* spp. was there a statistically significant difference in prevalence between 2018 and 2019 (P=0.033). In the research with 28 pediatric patients, *E. coli* (53.5%) was the most commonly detected gram-negative bacteria. The number of bacteria isolates was comparable to other studies conducted in various countries. *E. coli* was the most commonly isolated organism in South America, accounting for 39.7% of UTI cases, followed by *Enterococcus* spp. (11.5%).[27] Similarly, *E. coli* was the most isolated uropathogen in China, accounting for 66.01% of UTI cases, followed by *Enterococcus* spp.(5.91%).[28]

As bacterial resistance has increased in recent decades,[29-31] the isolates in this research demonstrated high resistance. In our study, 37.21% of the bacteria from the strain *E. coli*, 72.07% from the strain *Klebsiella* spp., 56.67% from the strain *Proteus* spp., 87.27% from the strain *P. aeruginosa*, and 66.07% from the strain *Acinetobacter* spp. showed resistance to more than one antibiotic.

Numerous organizations and programs are currently working to combat antibiotic resistance,[31] but the first step in obtaining a proper management and good control policy for decreasing the development of antibiotic resistance among microorganisms, particularly pathogens, is the evaluation and practical assessment of antibiotic resistance patterns among specific groups of patients in a country. Results of the antibiogram test for bacterial isolates recovered from UTI revealed that amikacin and imipenem were the most effective antimicrobials against the strains. E. coli, as the most common pathogen of UTIs in 2020, showed the most resistance to ampicillin (65.58%) and the least resistance to imipenem (1.09%) indicated. In 2018-2020, a trend of increased resistance of *E. coli* to ampicillin was registered from 37.41% in 2018 to 65.58% in 2020.

In the research on Tohid Hospital in Sanandaj, during 2013-2014, urinary pathogens were mostly resistant to ampicillin (64.15%) and trimethoprim/sulfamethoxazole (62.67%). Resistance was lowest to imipenem (0.7%) and amikacin (1.01%). The most prevalent pathogen of UTIs, *E. coli*, demonstrated the greatest resistance to ampicillin (43.87%) and the least resistance to nitrofurantoin (3.62%).

From the data of our research, we can conclude that the *E. coli*, *Proteus* spp., and *Klebsiella* spp. were the three commonly isolated microorganisms in the Peja region. Furthermore, most isolated bacterial microbes were resistant to antibiotics used in clinical practices in the country, which can be an emerging worry for a country's health control systems. It appears that administrators should use these medications with extreme caution and precision when treating UTIs and/or other infections. This requires health practitioners and policymakers to pay close attention to the resistance pattern in their clinical practice and policymaking processes. Data from this study can be used to control antibiotic susceptibility trends, create local antibiotic policies, and help clinicians in the rational choice of antibiotic therapy, thereby preventing indiscriminate antibiotic use.

Limitations of the study

Because only those who visited the health research laboratory through referral or on their own initiative were included in the study, this study may not reflect the general population of the Peja Region. The study included only antibiotics that have been examined in the regional health research laboratory.

Ethical considerations

Study approval was obtained from the Committee on Ethical Issues, Kosovo Doctors Chamber nr. 16/2022.

Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author:** *Sanije Hoxha–Gashi, Faculty of Medicine, University of Prishtina "Hasan Prishtina", National Institute of Public Health of Kosovo, Prishtina, Kosovo. E-mail: sanije.gashi@uni-pr.edu*