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Antibiotic Resistance Pattern of Uropathogens in Northeastern of Iran

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Urinary tract infection (UTI) is one of the most common bacterial infections worldwide. Inappropriate use of antibiotics in hospitalized UTI patients, and the consequent bacterial resistance, imposes high costs on health care settings. Therefore, this study aimed to investigate the prevalence of antimicrobial resistance in UTI patients hospitalized in the Ghaem University Hospital, Mashhad, Iran. Cross-sectional study with 28357 urine samples were collected from patients admitted in Ghaem University Hospital of Mashhad University Medical Sciences during the period from April 2017 to March 2020. For all samples, biochemical methods and the antimicrobial susceptibility testing was performed based on CLSI 2018. The collected data were analyzed using WHONET software. Out of 28357 urine samples, 3568 (12.58%) were confirmed as UTI. The prevalence of Gram negative and Gram positive bacteria was 77.42 and 22.58%, respectively. *Escherichia coli* (43.97%) and *Enterococcus faecalis* (15.27%) were found as the most prevalent species among Gram negative and Gram positive bacteria. *E. coli* showed the lowest rate of resistance to meropenem (3.7%) and amikacin (6.9%) and highly resistance rate was found for ampicillin (83.3%) and piperacillin (82.4%). Meropenem, amikacin, linezolid, and nitrofurantoin showed better *in vitro* efficacy against urinary tract pathogens compared with other antibiotics. Therefore, despite universal guidelines, we recommend that empirical antibiotic selection should be done based on the local prevalence of bacterial organisms and antibiotic sensitivities.

Key words: Urinary tract infection, uropathogens, antibacterial susceptibility pattern, bacterial infections, Gram-negative bacteria, *Escherichia coli*.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases in both hospitals and community (Sowmya, 2019). Hospital-acquired UTIs occur within 48 h after hospitalization or three days after discharge (Folliero et al., 2020). Moreover, UTI imposes considerable costs on the healthcare system worldwide (Wawrysiuk et al., 2019). UTI accounts for nearly 7 million visits in the outpatient office and 1 million visits in

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the emergency department, resulting in 100,000 hospitalizations and at least *$6* billion in health care (Goel and Mukherjee, 2016; Ahmed et al., 2019; Shafrin et al., 2022). UTI includes a variety of clinical conditions ranging from asymptomatic bacteriuria to severe kidney infection with resultant sepsis. UTI can affect all people and ages, with different risk factors including race, genetic, age, gender, sexual activity, nocturnal enuresis, and circumcision in boys (Mohammed et al., 2016). However, UTI is more common in women due to sexual activity, pregnancy, and short urethral anatomy (Fallah et al., 2019). Approximately 50 to 60% of women experience UTI at least once in their lifetime (Ahmed et al. 2019).

Gram-negative bacteria usually account for 70-90% of UTI cases, in contrast, Gram-positive bacteria cause only 10% of the cases (Zahedi et al., 2019). Among Gram-negative bacteria Enterobacteriaceae, especially *Escherichia coli* and *Klebsiella* species are the most common, while Gram-positive bacteria, such as *Enterococcus* species, *Staphylococcus aureus* and Coagulase-Negative *Staphylococci* (CoNS) are less frequent (Akbar et al., 2017).

The World Health Organization (WHO) estimates about 20 to 50% of antibiotics are misused in the community (Alanazi, 2018). Consequently, the decreased antibiotic efficacy associates with delay in effective treatment, increased risk of renal scarring, impaired renal function, early hypertension, and chronic renal failure later in life (Ladhani and Gransden, 2003). Efficacious antibiotic therapy depends on the antimicrobial susceptibility testing, severity of the symptoms and potential complications of the patient (Mulder et al., 2019).

Monitoring and controlling the spread of antimicrobial resistance is particularly challenging for clinical microbiologists and infectious disease specialists in developing countries because of multiple factors, including lack of follow-up systems, limited resources, weak adherence to infection control measures, and careless use of antibiotics (Ntirenganya et al., 2015). Furthermore, some studies reported that antibiotic resistance in previous infections can be used to predict resistance to future infections (Yelin et al., 2019).

Controlling antimicrobial resistance is one of the most controversial challenges in public health. Moreover, monitoring of antibiotic resistance patterns for uropathogens may be advantageous due to selection of an optimal antibiotic therapy plan. Therefore, this study aimed to determine the prevalence and antibiotic resistance profiles of bacteria responsible for UTIs.

METHODOLOGY

Sample collection

Cross-sectional study and the retrospective analysis were carried out at Department of Microbiology, Ghaem University hospital, Mashhad, Iran, from April 2017 to March 2020, 28357 urine samples were collected in sterile containers by clean-catch midstream urine samples of hospitalized patients.

Laboratory methods

According to standard urine culture method, urine samples were cultured on 5% sheep blood agar and MacConkey agar using calibrated loops. The plates were incubated at 37°C (± 1°C) for 24 h. Then, the colonies were counted, Gram staining was performed for appropriate samples (Monsen and Monsen, 2017). Subsequently, appropriate biochemical tests were selected based on the Gram staining findings. The biochemical tests included lactose consumption, and Enterobacteriaceae diagnostic kit (including Triple sugar iron agar (TSI), SIM, urease test, phenyl alanine decarboxylase, etc.) for Gram negative isolates, and mannitol consumption, coagulase test, and DNase for Gram positive isolates. Next, antibiotic susceptibility testing was performed on Mueller-Hinton agar, using Kirby-Bauer's disk diffusion method according to guidelines of the Clinical and Laboratory Standards Institute.

Data analysis

The data were analyzed using WHONET software 5.6 provided by the World Health Organization for antimicrobial resistance surveillance of uropathogenic bacteria.

RESULTS

Out of 28357 urine samples, 3568 (12.58%) samples were confirmed as UTI. As demonstrated in Figure 1, UTI cases were more frequent among the middle-aged adults (41.45%) and females (56.17%) as compared to children (6.78%) and males (43.83%) patients. Following the middle-aged adults, UTI was detected in elderly patients (25.22%). Moreover, the frequency of UTI among women was higher in comparison to men. The prevalence of UTI among the middle-aged adults was significantly higher than other groups (p-value< 0.05).

The frequency of Gram-negative and -positive bacteria was 77.42 and 22.58%, respectively. According to Figure 2, among 3568 isolates, the most prevalent species were *E. coli* (N=1569, 43.97%), *Klebsiella pneumoniae* (N=546, 15.3%), *Enterococcus faecalis* (N=545, 15.27%), and *Pseudomonas aeruginosa* (N=278, 7.8%).

Based on Figure 2a, *E. coli* (43.97%), *K. pneumonia* (15.3%), and *P. aeruginosa* (7.8%) were the most prevalent Gram-negative bacteria which caused UTI. Moreover, frequency of Gram-positive bacteria was observed as follows: *E. feacalis* (15.27%), *Staphylococcus epidermidis* (2.18%), *S. aureus* (1.79%), and *Staphylococcus saprophyticus* (1.23%) (Figure 2b).

According to Table 1, the most prevalent resistance was observed against β-lactams and cephems. The least resistance was detected against meropenem, amikacin, and piperacillin-tazobactam. The highest and lowest resistance rates were observed against ampicillin (83.3%) and meropenem (3.7%) among *E. coli* isolates. While, the lowest resistance rates among *P. aeruginosa*, *K. pneumonia*, and *Acinetobacter* species were detected

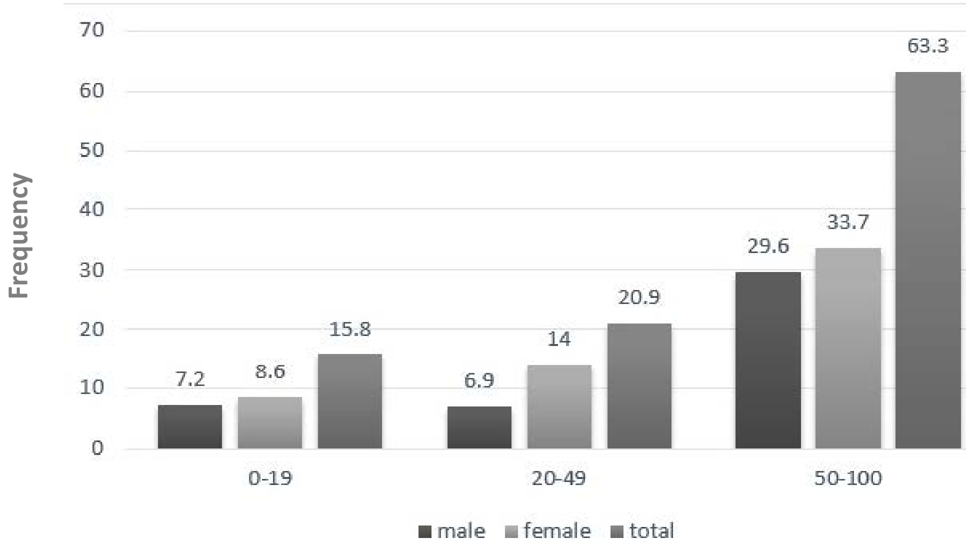

Figure 1. Frequency distribution of UTI between different age groups.
Source: Author

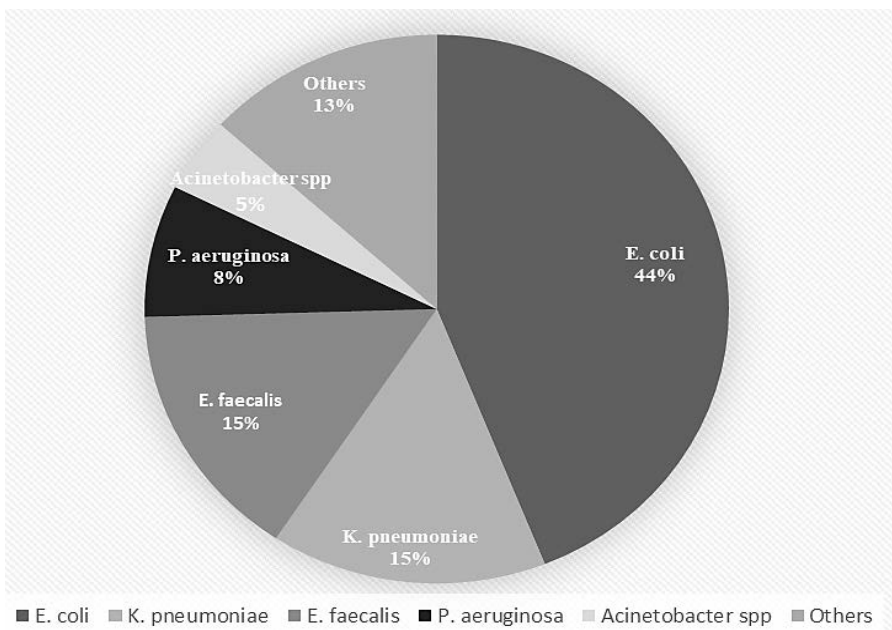

Figure 2. Frequency of uropathogenic organisms isolated from urine samples.
Source: Author

against piperacillin-tazobactam (50.71%), amikacin (33.15%), and trimethoprim-sulfamethoxazole (49.68%), respectively.

Based on the Table 2, the pattern of antimicrobial resistance among Gram-positive uropathogenic bacteria revealed the least frequent rates of resistance against linezolid. Interestingly, the most prevalent resistance was against azithromycin (88.25%) and erythromycin (89.17%). Moreover, the frequency of Methicillin-resistant *S. aureus* (MRSA), Methicillin-Resistant *S.epidermidis* (MRSE), and vancomycin-resistant enterococcus (VRE) among uropathogenic isolates was 0.9, 2.29, and 5.7%, respectively (Figure 3). As depicted in Figure 3, out of 3568 UTI isolates, 49.43% MDR, 30.4% XDR, and 15.47% PDR isolates were detected.

In general, among all the Gram-negative isolates the

Table 1. Antibiotic resistance of uropathogenic Gram-negative bacteria against tested antibiotics.

Antibiotic disks	Resistance rate, %							
	Escherichia coli **N=1569**	***Klebsiella pneumonie*** **N=546**	***Pseudomonas aeruginosa*** **N=278**	***Acinetobacter* spp. N=157**	***Enterobacter* spp. N=96**	***Klebsiella oxytoca*** **N=59**	***Proteus* spp. N=36**	***Citrobacter* spp. N=21**
Amikacin	108 (6.88)	181 (33.15)	195 (70.14)	116 (73.88)	12 (12.5)	15 (25.42)	2 (5.55)	2 (9.52)
Ceftazidime	765 (48.75)	364 (66.66)	240 (86.33)	138 (87.89)	40 (41.66)	31 (52.54)	6 (16.66)	6 (28.57)
Cefepime	808 (51.49)	345 (63.16)	223 (80.21)	132 (84.07)	42 (43.75)	35 (59.32)	2 (5.55)	9 (42.85)
Gentamicin	462 (29.44)	278 (50.91)	176 (63.3)	117 (74.52)	29 (34.37)	15 (25.42)	3 (8.33)	9 (42.85)
Imipenem	331 (21.09)	221 (40.47)	206 (74.1)	107 (68.15)	11 (11.45)	14 (23.72)	3 (8.33)	8 (38.09)
Ciprofloxacin	924 (58.89)	303 (55.49)	209 (75.17)	129 (82.16)	45 (46.87)	21 (35.59)	12 (33.33)	9 (42.85)
Meropenem	58 (3.69)	247 (45.23)	196 (70.5)	105 (66.87)	10 (10.41)	13 (22.03)	2 (5.55)	2 (9.52)
Cefazolin	1081 (68.89)	417 (76.37)	278 (100)	147 (93.63)	79 (82.29)	21 (66.7)	10 (27.77)	11 (52.38)
Ceftriaxone	1026 (65.39)	384 (70.32)	227 (81.65)	142 (90.44)	65 (67.7)	39 (61.1)	9 (25)	21 (100)
Ampicillin	1306 (83.23)	518 (94.87)	278 (100)	150 (95.54)	83 (86.45)	53 (89.83)	18 (50)	17 (80.95)
Cefotaxime	953 (60.73)	362 (66.3)	242 (87.05)	143 (91.08)	65 (67.7)	39 (66.1)	7 (19.44)	5 (23.8)
Ceftazidime/Clavulanic acid	252 (16.06)	250 (45.78)	249 (89.56)	122 (77.7)	22 (22.91)	8 (13.55)	7 (19.44)	7 (33.33)
Cefixime	991 (63.16)	366 (67.03)	278 (100)	145 (92.35)	70 (72.91)	31 (52.54)	18 (50)	8 (38.09)
Piperacillin	1292 (82.34)	416 (76.19)	144 (51.79)	115 (73.24)	79 (82.29)	42 (71.18)	0 (0)	15 (71.42)
Nitrofurantoin	127 (8.09)	284 (52.01)	269 (96.76)	136 (86.62)	29 (30.2)	13 (22.03)	25 (69.44)	0 (0)
Colistin	227 (14.46)	71 (13)	6 (2.15)	22 (14.01)	22 (22.91)	15 (25.42)	22 (61.11)	11 (52.38)
Trimethoprim/Sulfamethoxazole	1156 (73.67)	379 (69.41)	278 (100)	78 (49.68)	32 (33.33)	44 (74.57)	36 (100)	7 (33.3)
Piperacillin/Tazobactam	150 (9.56)	240 (43.95)	141 (50.71)	116 (73.88)	11 (11.45)	20 (33.89)	0 (0)	0 (0)

Source: Author

highest resistance was related to ampicillin (86.2%) followed by cefazolin (79%), piperacillin (78.2%), trimethoprim/sulfamethoxazole (71.3%), ceftriaxone (68.1%), and cefixime (66.7%). The best antimicrobials for Gram-negative organisms were amikacin (74.8%) and meropenem (73.2%). However, Gram-positive isolates also indicated resistance to azithromycin (89.17%) followed by erythromycin (88.25%). In contrast, the sensitivity pattern of antimicrobial for Gram-positive organisms was linezolid (93.3%) and nitrofurantoin (86.3%) (Regardless of the type of bacteria).

DISCUSSION

This study provides valuable data to compare and monitor the status of antimicrobial resistance among uropathogens to select the best empirical antibiotic therapy. Uropathogen isolates in this study were similar to those of many other studies. Similarities and differences in the type and spreading of bacterial pathogens due to different environmental conditions and host factors (Salleh et al., 2017). The frequency of the UTIs among women and men was 56.17 and 43.83%, respectively. As described in many studies from Pakistan, Iran, and India, the ratio of UT-infected women was significantly more than the men (50-60%), which was in accordance with this study (Prakash and Saxena, 2013, Ghanbari et al., 2017; Akbar et al., 2017). Moreover, the frequency of UTI in middle-aged women was higher (24.5%) than other age groups. According to Medina and Castillo-Pino (2019) the burden of UTI in women increases with age, and as demonstrated in Figure 1, a significant growth in UTI was observed.

Table 2. Antibiotic resistance of uropathogenic Gram-positive bacteria against tested antibiotics.

Antibiotic disks	Resistance rate, %				
	Enterococcus faecalis **N=545**	***Staphylococcus epidermidis*** **N=78**	***Staphylococcus aureus*** **N=64**	***Staphylococcus saprophyticus*** **N=44**	**Others (N=75)**
Gentamicin	332 (60.91)	14 (17.94)	7 (10.93)	13 (29.54)	32 (42.66)
Vancomycin	206 (37.79)	10 (12.82)	3 (4.68)	22 (50)	31 (41.33)
Linezolid	29 (5.32)	-	-	-	7 (9.33)
Ampicillin	278 (51)	-	-	-	43 (57.33)
Penicillin G	397 (72.84)	-	-	-	61 (81.33)
Ciprofloxacin	448 (82.2)	34 (43.58)	22 (34.37)	22 (50)	42 (56)
Erythromycin	486 (89.17)	57 (73.07)	48 (75)	31 (70.45)	65 (86.66)
Azithromycin	481 (88.25)	59 (75.64)	47 (73.43)	38 (86.36)	30 (40)
Doxycycline	332 (60.91)	33 (42.3)	30 (46.87)	31 (70.45)	37 (49.33)
Clindamycin	456 (83.66)	41 (52.56)	36 (56.25)	33 (75)	55 (73.33)
Cefoxitin	439 (80.55)	50 (64.1)	35 (54.68)	32 (72.72)	53 (70.66)
Tetracycline	443 (81.28)	-	-	-	41 (54.66)
Nitrofurantoin	65 (11.92)	7 (8.97)	6 (9.37)	7 (15.9)	9 (12)

Source: Author

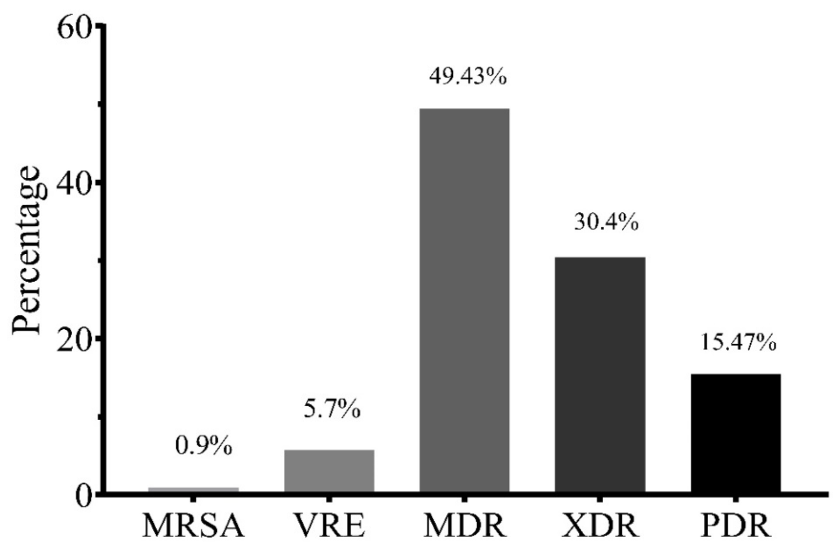

Figure 3. The frequency of MRSA, MRSE, and VRE among uropathogenic isolates.
Source: Author

Based on the Figure 2, *E. coli* was the most prevalent microorganism causing UTI with 43.97%. Different studies from Iran, Saudi Arabia and Germany reported *E. coli* as the most frequent cause of UTI (Jasemi et al., 2011; Alanazi, 2018, Rajabnia et al., 2019). Although Habibi Asl and Bischoff studies described *E. coli* as the most common agent of UTI, the reported prevalence (68.9 and 64%, respectively) was much more than the current study (Asl et al., 2017; Bischoff et al., 2018). These differences may be related to the treatment protocol and the antibiotic consumption in that area. Medina and Castillo-Pino 2019 described that *K. pneumoniae* and *E. faecalis* were at the two next stages of frequency, which are consistent with this study. Also, Asl et al. (2017) and Kitagawa et al. (2018) reported the same data on UTI prevalence among hospitalized patients. Among Gram positive bacteria, *E. faecalis* and *S. saprophyticus* showed the higher and lowest frequency (15.27 and 1.23%), respectively. Gajdács et al. reported *Enterococcus* as the most prevalent genus causing UTI (Gajdács et al., 2020). However, a review by Kline and Lewis (2016) showed *S. saprophitycus* as the most prevalent Gram-positive bacteria causing UTI. Moreover, 5.7% of UTI isolates were confirmed as VRE, which was higher than the previous studies of Gajdács et al. (2020) with a prevalence of 0.16%. However, higher frequencies were reported in other studies (Toner et al., 2016; Pontefract et al., 2020). MRSA isolates among UTI cases were observed as 0.9%, which was lower than other studies with 22.5 and 42.5% (Gajdács et al., 2020; Mitiku et al., 2021). The differences between the current study and aforementioned studies may be due to the population and the geographic centers the studies were conducted, as well as sample size and type of technique were used in detection and diagnosis.

As demonstrated in Table 2, Gram-positive bacteria isolated in this study were highly resistant to macrolides (erythromycin and azithromycin). The least resistance was observed against linezolid (0-9.33%) among different Gram-positive species. In contrast to this study, the highest rate of resistance was observed against fluoroquinolones and trimethoprim-sulfamethoxazole (Linhares et al., 2013). Moreover, the most prevalent antibiotic resistance was detected against ampicillin, ciprofloxacin, and tetracyclines (Toner et al., 2016; Looney et al., 2017; Gajdács et al., 2020). The contrast may be due to the differences among the health settings programs of antibiotic administration policy and the antibiotic's availability in each region. To illustrate, in a 4 years period (2008-2012) the highest resistance detected in *S. aureus* was detected against azithromycin. While in the next 5 years period, the resistance to this antibiotic decreased significantly (Gajdács et al., 2020).

In Aghamahdi et al. (2013) study, 95% of urinary pathogens were resistance to ampicillin and 69.4% to co-trimoxazole but resistance to aminoglycosids, ceftriaxone and ciprofloxacin was much less. In the previous study, Rajabnia et al. (2019) reported the highest resistance to cefixime (92.51%), ceftriaxone (52.54%) and cotrimoxazole (44.48%) and the highest susceptibility to imipenem (96.11%), nitrofurantoin (91.67%) and ceftazidime (88.28%). Isolated uropathogens, in Mahmoud et al. (2016) study, were highly sensitive to amikacin, imipenem and meropenem.

Understanding the antimicrobial resistance patterns in *E. coli*, as the most frequent uropathogen is significant to choose an empirical antibiotic therapy. As shown in Table 1, *E. coli* showed high level of resistance to ampicillin (83.3%) and piperacillin (82.4%) followed by trimethoprim/sulfamethoxazole (73.7%). The most effective anti-bacterial agents in our study were amikacin, meropenem, nitrofurantoin and piperacillin/tazobactam for *E. coli* (91-96%).

Akbar et al. (2017) from Pakistan has reported that imipenem (92.9%) and amikacin (82.5%) were the most effective against *E. coli* isolates. Another study has reported *E. coli* strains showed the highest sensitivity to gentamicin (56.59) and ceftriaxone (34.5%) (Zare et al., 2018). Another study from Nepal showed 87% of *E. coli* were resistant to ampicillin, 62% ceftriaxone and nitrofurantoin (5% resistance) are the most effective drugs for Gram-negative bacilli (Shrestha et al., 2019).

According to the results of antibiogram test in this study, among all the Gram-negative isolates, highest resistance was related to ampicillin (86.2%) followed by cefazolin (79%), piperacillin (78.2%), trimethoprim/ sulfamethoxazole (71.3%), ceftriaxone (68.1%), and cefixime (66.7%). The best antimicrobials for Gram-negative organisms were colistin (84.1%), amikacin (74.8%) and meropenem (73.2%).

In conclusion, meropenem, amikacin, linezolid, and nitrofurantoin showed better *in vitro* efficacy against urinary tract pathogens compared with other antibiotics. Therefore, despite universal guidelines, we recommend that empirical antibiotic selection should be done based on the local prevalence of bacterial organisms and antibiotic sensitivities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATION

UTI, Urinary tract infections; **CONS**, Coagulase-Negative Staphylococci; **MRSA**, Methicillin-resistant Staphylococcus aureus; **MRSE**, Methicillin-Resistant Staphylococcus epidermidis; **VRE**, vancomycin-resistant enterococcus; **WHO**, World Health Organization; **MDR**, Multidrug resistant; **XDR**, Extensively drug-resistant; **PDR**, Pandrug-resistant.

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