Journal of Advances in Medical and Biomedical Research | ISSN:2676-6264

Bacterial Urinary Tract Infections in North West of Iran: A Cross- Sectional Study

Erfan Fakheri[1], Niloufar Kazemi[1], Narges Moradi[1,2], Bahman Mirzaei[3,4]*

1. *Student Research Committee, Zanjan University of Medical Sciences, Zanjan, Iran*
2. *Dept. of Life Technologies, University of Turku, Turku, Finland*
3. *Dept. of Bacteriology, Pasteur Institute of Iran, Tehran, Iran*
4. *Dept. of Medical Microbiology and Virology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran*

Article Info

doi 10.30699/jambs.31.148.464

Received: 2022/08/09;
Accepted: 2022/00/20;
Published Online: 29 Oct 2023;

Use your device to scan and read the article online

Corresponding Information:
Bahman Mirzaei,
Dept. of Bacteriology, Pasteur Institute of Iran, Tehran, Iran

E-Mail: dr.bahman.m@gmail.com

ABSTRACT

Background & Objective: Bacteria play a major role in urinary tract infections (UTIs); therefore, it is necessary to be aware of their regional prevalence and the causative pathogens for better prognosis and rapid treatment in clinical settings. This study aims to evaluate the prevalence of bacterial isolates involved in UTI samples and their antibiotics resistance pattern.

Materials & Methods: In this cross-sectional study, bacterial infections from 4214 urine samples were analyzed from December 2016 to December 2018. After biochemical tests, disk diffusion susceptibility procedures were performed on all positive clinical cultures, according to CLSI guidelines. The obtained data were sorted and statistically analyzed by SPSS 26.

Results: Out of 3582 suspected UTIs samples, 2006 (56%) were females and 1576 (44%) males in the 0-99 years old age range and mainly consisting of middle-aged and elderly patients (62.2%). *Escherichia coli* (53.43%) and *Staphylococcus epidermidis* (15.99%) were the most frequent isolates. Among gram negative bacteria, nitrofurantoin and among gram- positive**,** vancomycin represented the lowest resistance rates at 25.27% and 26.74% respectively. Piperacillin showed the least efficacy with a resistance rate of 76.04%, followed by cefazolin with a 74.94% resistance rate. In gram positive bacteria, vancomycin and gentamicin showed more promise with respective resistance rates of 19.34% and 27.34%. The highest resistance was associated with ampicillin (68.61%) and Trimethoprim/Sulfamethoxazole (66.06%).

Conclusion: Alarming resistance rates were observed in ampicillin and piperacillin, which should be taken into account in therapy guidelines in this area. Prevalence of resistant strains can be avoided by developing appropriate healthcare policies and community awareness.

Keywords: Antimicrobial Resistance, Bacteria, Hospital-acquired Infection, Urinary Tract Infection

Introduction

Nosocomial infections or healthcare-associated infections (HAIs) are infections that hospitalized patients acquire throughout their period of stay. Symptoms of the disease may appear during the hospitalization or after discharge from the hospital (1). Infections that appear after 48 to 72 hours are usually classified as nosocomial, and if the infection occurs less than 48 hours of hospitalization, it is likely that the patient might have obtained the infection during the administration process and may have been in the common phase of the disease (2). A recent meta-analysis conducted by Mohammadi *et al.,* from 2001 to 2017, reported a 4.6% overall prevalence rate of HIAs in Iranian hospitals (3). A 2014 CDC report including 183 US hospitals and 11,282 hospitalized patients indicated that 4% of the patients suffered from one or more HAIs (4). This results in a rise in costs, length of recovery, disabilities and even death amongst patients and attaches great importance to development of regionally studied and validated diagnostic and therapeutic guidelines for these infections.

Bacteria are microorganisms that are found in various environments, especially in clinical settings. The ability of bacteria to cause hospital outbreaks and epidemics is indicative of the importance of these microorganisms. Bacteria are mainly labeled as opportunistic pathogens and can create various acquired infections in clinical settings including bacteremia, meningitis, surgical wound infections, urinary tract infections (UTIs), hospital-acquired pneumonia, etc. (5). UTIs are bacterial infections that affect various parts of the urinary tract. Physiologic and anatomic differences have made UTIs more prevalent among women than men; however, the prevalence rate is similar in elderly males and females (6). Infections of the lower urinary tract and the upper urinary tract are referred to as cystitis (bladder infection) and pyelonephritis (kidney infection), respectively (7). Symptoms of the lower urinary tract infections include frequent urination accompanied by pain (dysuria), while symptoms of pyelonephritis include fever, shivering and flank pain in addition to the symptoms of lower urinary tract infections (8). The main cause of both types of infections is uropathogenic *Escherichia coli*, a gram negative rod-shaped bacterium from the Enterobacteriaceae family (9). The increasing antibiotic resistance and the emergence of multidrug-resistant (MDR) and extensively-drug resistant (XDR) *E. coli* strains have led to a global health crisis. In most of the infections, the hospital environment is the source and reservoir and, in several studies, it has been observed that environmental pollution has been the source of sudden epidemics caused by bacterial infections (1). Bacteria play an important role in UTIs; therefore, it is necessary to be aware of their prevalence and the causative pathogens for better prognosis, rapid treatment and elimination of the infectious agents in clinical settings. This study aims to evaluate the prevalence of bacterial isolates involved in UTI samples and their antibiotics resistance pattern.

Materials and Methods

Sample Collection and Culture

A cross-sectional study was designed to analyze bacterial infections in a total number of 3582 urine samples collected from ambulatory and hospitalized patients in Valie-Asr hospital in Zanjan, Iran, from December 2016 to December 2018. Samples were initially cultured on MacConkey agar and blood agar (Lioflchem, Italy), according to standard bacteriological protocols and incubated at 37°C for 24 hours (10).

Identification of Bacterial Isolates

Bergey̓s microbiology book guidelines were applied for isolate identification (10). Gram staining and conventional media and biochemical tests including catalase, oxidase, Sulfide Indole Motility (SIM), Triple Sugar Iron Agar (TSI), Methyl Red (MR)/Voges-Proskauer (VP), citrate, sensitivity to specific antibiotic disks, Mannitol Salt Agar, urease, Dnase, etc. (Merck, Germany) were used to confirm the growth of the bacteria.

Antibiotic Susceptibility Testing

Disk diffusion susceptibility tests were performed on all positive clinical cultures, according to Clinical Laboratory Standard Institute (CLSI) guidelines (11) using the disks (BD BBLTM Sensi DiscTM) containing amikacin (AN) (30 µg), ceftazidime (CAZ) (30 µg), cephalexin (30 µg), levofloxacin (LEV) (5 µg), piperacillin-tazobactam (PI 10 µg) ciprofloxacin (CP) (5 µg), imipenem (IMP) (10 µg), meropenem (MEN) (10 µg), gentamycin (GM) (10 µg), ampicillin (10 µg), ceftriaxone (CRO) (30 µg), cefotaxime (CTX) (30 µg), trimethoprim/sulfamethoxazole (SXT) (25 [1.25/23.75] µg), cefazolin (CZ), cefepime (FEP) (30 µg), nitrofurantoin (FM) (300 µg), oxacillin (CX) (30 µg), and vancomycin (30 µg) (only for gram positive bacteria) (11). American Type Culture Collection (ATCC) standard reference strains (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213) were used as controls.

Statistical Analysis

The obtained data were sorted and statistically analyzed in SPSS 26 (SPSS Inc., Chicago, IL, USA). Pearson's chi-square test was used for variable comparison in independent groups and P-values less than 0.05 were considered significant.

Ethics approval and consent to participate

This study complies with ethical principles and the standards for conducting clinical research and is approved by The Ethics Committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1398.370).In the current study, all ethical guidelines including Ethics and Consent to participate from the parents have been collected.

Results

From December 2016 to December 2018, a total number of 6299 urinary, respiratory tract, blood, and stool culture tests were performed for the patients who referred to Valie-Asr Hospital Laboratory in Zanjan, Iran, including 3168 (50.3%) males and 3131 (49.7%) females. Urine culture was the most frequently requested culture (n=3582, 56.87%), followed by sputum (n=1058, 16.8%), blood (n=854, 13.4%) and stool (n=182, 2.9%) cultures. In this study, we analyzed microbial cultures of patients with bacterial infections of the urinary tract and the associated antimicrobial resistance patterns. The study population consisted of a total number of 3582 patients with positive urine cultures (85%), including 2006 (56%) females and 1576 (44%) males in the 0-99 age range. The study population mainly consisted of middle-aged and elderly patients (n=2228, 62.2%), followed by young adults (n=1021, 28.5%). Positive urine cultures were the least frequent among adolescents and children (n=333, 9.3%). The average age of the study population was 63.4 years. With 1275 positive bacterial growth results, the emergency ward had the highest positive culture ratio (35.6%), followed by the following wards: internal (17.8%, n=638), neurosurgery (16.9%, n=605), intensive care unit (11.8%, n=423), infectious diseases (10.4%, n=373), cardiology (3.7%, n=133), surgery (1.6%, n=57), coronary care unit (0.9%, n=32) oncology (0.7%, n=25), and ophthalmology (0.6%, n=21).

According to urine cultures results, *E. coli* and *Staphylococcus epidermidis* were the most frequently isolated gram negative and gram-positive bacteria from the urine samples with isolation rates of 53.43% and 15.99%, respectively. Table 1 reveals information on the prevalence of the isolated bacteria.

Table 1. Prevalence of isolated bacteria from the urine samples of a total number of 3582 patients with positive urine cultures.

		Isolation frequency (N=3582)
Gram Negative	*Escherichia coli*	53.43% (n=1914)
	Serratia marcescens	5.38% (n=193)
	Pseudomonas aeruginosa	4.52% (n=162)
	Klebsiella pneumoniae	4.15% (n=149)
	Acinetobacter baumanni	0.3% (n=11)
	Citrobacter freundii	0.16% (n=6)
	Morganella morganii	0.11% (n=4)
	Proteus mirabilis	0.08% (n=3)
Gram Positive	*Staphylococcus epidermidis*	15.99% (n=537)
	Group D Streptococci	7.7% (n=279)
	Staphylococcus saprophyticus	6.19% (n=222)
	Lactobacillus acidophilus	1.2% (n=44)
	Viridans streptococci	1% (n=36)
	Staphylococcus aureus	0.6% (n=22)

Among gram negative bacteria, nitrofurantoin and vancomycin represented the lowest resistance rates at 25.27% and 26.74% respectively. Piperacillin showed the least efficacy with a resistance rate of 76.04%, followed by cefazolin which had a resistance rate of 74.94%. Antibiotic susceptibility results of the isolated gram-negative bacteria are demonstrated in Table 2.

Table 2. Antibiotic susceptibility pattern of the gram-negative bacteria isolated from 2442 urine samples of the clinical origin and the associated resistance rates.

		E. coli	S. marcescens	P. aeruginosa	K. pneumonia	A. bumannii	C. freundii	M. morganii	P. mirabilis	Total (N)	Resistance Rate (%)
	Number (n)	1914	193	162	149	11	6	4	3	2442	
Amikacin	Sensitive	1178	19	47	63	2	4	3	2	1318	30.18
	Intermediate	353	3	2	24	2	1	1	1	387	
	Resistant	383	171	113	62	7	1	0	0	737	
Gentamycin	Sensitive	1187	15	50	49	6	3	0	3	1313	42.67
	Intermediate	76	3	3	2	1	1	1	0	87	
	Resistant	651	175	109	98	4	2	3	0	1042	
Imipenem	Sensitive	1417	19	36	83	2	0	4	2	1563	30.63
	Intermediate	95	17	2	14	1	2	0	0	131	
	Resistant	402	157	124	52	8	4	0	1	748	
	Sensitive	1531	10	130	18	1	1	2	0	1693	25.27

		E. coli	S. marcescens	P. aeruginosa	K. pneumonia	A. baumannii	C. freundii	M. morganii	P. mirabilis	Total (N)	Resistance Rate (%)
Nitrofurantoin	Intermediate	115	2	3	12	0	0	0	0	132	
	Resistant	268	181	29	119	10	5	2	3	617	
Trimethoprim/Sulfamethoxazole	Sensitive	670	11	4	31	0	2	2	2	722	67.94
	Intermediate	57	2	1	1	0	0	0	0	61	
	Resistant	1187	180	157	117	11	4	2	1	1659	
Ciprofloxacin	Sensitive	804	19	36	32	0	4	4	3	902	62.57
	Intermediate	4	2	5	1	0	0	0	0	12	
	Resistant	1106	172	121	116	11	2	0	0	1528	
Cefepime	Sensitive	957	10	47	47	5	4	2	1	1073	52.78
	Intermediate	57	2	7	11	0	0	2	1	80	
	Resistant	900	181	108	91	6	2	0	1	1289	
Ceftriaxone	Sensitive	756	10	17	38	1	2	2	2	828	64.62
	Intermediate	17	2	11	3	3	0	0	0	36	
	Resistant	1141	181	134	108	7	4	2	1	1578	
Piperacillin	Sensitive	459	10	37	21	1	0	2	3	533	76.04
	Intermediate	38	2	4	3	2	2	1	0	52	
	Resistant	1417	181	121	125	8	4	1	0	1857	
Cefazolin	Sensitive	511	4	14	19	0	2	1	2	553	74.94
	Intermediate	54	1	0	2	0	1	0	1	59	
	Resistant	1349	188	148	128	11	3	3	0	1830	
Ceftazidime	Sensitive	924	7	47	50	2	3	0	2	1035	51.68
	Intermediate	118	3	11	12	0	1	0	0	145	
	Resistant	872	183	104	87	9	2	4	1	1262	
Levofloxacin	Sensitive	840	14	39	61	2	2	4	2	964	57.82
	Intermediate	54	3	3	3	2	0	0	1	66	
	Resistant	1020	176	120	85	7	4	0	0	1412	
Ampicillin	Sensitive	717	19	41	47	6	2	2	3	837	53.77
	Intermediate	239	7	1	43	1	1	0	0	292	
	Resistant	958	167	120	59	4	3	2	0	1313	
Cefoxitin	Sensitive	1311	8	21	89	5	5	2	2	1443	39.60
	Intermediate	19	3	5	2	1	1	1	0	32	
	Resistant	584	182	136	58	5	0	1	1	967	

Among gram positive bacteria, vancomycin and gentamicin showed more promise with respective resistance rates of 19.34% and 27.34%. The highest resistance was associated with ampicillin (68.61%) and Trimethoprim/Sulfamethoxazole (66.06%). Antibiotic susceptibility results of the isolated gram-positive bacteria are demonstrated in Table 3.

Table 3. Antibiotic susceptibility pattern of the gram-positive bacteria isolated from 1096 urine samples of the clinical origin and the associated resistance rates.

		S. epidemidis	Group D Streptococci	S. saprohyticus	Viridans streptococci	S. aureus	Total (N)	Resistance rate (%)
	Number (n)	537	279	222	36	22	1096	
Amikacin	Susceptible	326	93	57	5	8	489	46.99
	Intermediate	74	10	7	1	0	92	
	Resistant	137	176	158	30	14	515	
Gentamicin	Susceptible	397	104	145	30	19	695	27.83
	Intermediate	23	57	13	2	1	96	
	Resistant	117	118	64	4	2	305	
Imipenem	Susceptible	254	80	109	24	12	479	50.91
	Intermediate	4	40	8	6	1	59	
	Resistant	279	159	105	6	9	558	
Nitrofurantoin	Susceptible	388	224	130	27	18	787	24.82
	Intermediate	16	11	7	2	1	37	
	Resistant	133	44	85	7	3	272	
Trimethoprim/ Sulfamethoxazole	Susceptible	191	84	43	20	12	350	66.06
	Intermediate	12	1	8	1	0	22	
	Resistant	334	194	171	15	10	724	
Ciprofloxacin	Susceptible	219	73	65	18	12	387	59.58
	Intermediate	24	11	14	2	5	56	
	Resistant	294	195	143	16	5	653	
Piperacillin	Susceptible	260	78	147	4	5	494	51.55
	Intermediate	8	16	7	3	3	37	
	Resistant	269	185	68	29	14	565	
Cefepime	Susceptible	281	93	58	5	17	454	54.38
	Intermediate	25	12	6	0	3	46	
	Resistant	231	174	158	31	2	596	
Vancomycin	Susceptible	517	176	119	31	19	862	19.34
	Intermediate	12	6	3	0	1	22	
	Resistant	8	97	100	5	2	212	
Cefazolin	Susceptible	329	42	34	17	18	440	57.12
	Intermediate	9	8	7	4	2	30	
	Resistant	199	229	181	15	2	626	
Oxacillin	Susceptible	134	164	22	12	15	347	63.32
	Intermediate	11	33	4	6	1	55	

		S. epidemidis	Group D Streptococci	S. saprohyticus	Viridans streptococci	S. aureus	Total (N)	Resistance rate (%)
	Resistant	392	82	196	18	6	694	
Ampicillin	Susceptible	119	93	86	14	7	319	68.61
	Intermediate	6	14	3	1	1	25	
	Resistant	412	172	133	21	14	752	
Levofloxacin	Susceptible	253	159	93	4	9	518	51.09
	Intermediate	10	2	4	2	1	19	
	Resistant	274	118	125	30	13	560	

Discussion

A total number of 3582 ambulatory and hospitalized patients with bacterial UTI were included in our study. The overall prevalence of UTI was significantly high (85%, $P<0.001$) among the patients with a urine culture request compared to several similar studies, which reported overall UTI prevalence of 12.1% to 45.32% **(12-15)**. Even though this prevalence variation can be due to geographical and environmental factors, diagnostic methods applied in the study and the characteristics of the included patients such as personal hygiene standards and diet **(15)**, high prevalence ratio in our study raises the concern of sufficiency of the quantity of microbiologic tests requested by the physicians in this clinical setting in Zanjan, Iran from 2016 to 2018.

Similar to other studies in this timeframe and as expected from the general epidemiology of UTIs **(14)**, the female patients consisted the majority of the population in our study. Furthermore, a significant increase was observed in the prevalence of UTI with age, given that most of the patients were from the middle-aged and the elderly group ($P=0.016$). This can be explained by higher number of hospital admission, longer duration of stay and previous antibiotic therapy in the elderly. UTI incidence in women over 60 was 1.7 times higher than the overall incidence in females, putting this group at the highest risk. This result is similar to that of a study by Alós, which reported 20% and 11% UTI prevalence rates in women over 65 years and in the overall population, respectively **(16)**.

Gram-negative bacteria were significantly more prevalent (68%) than gram positive-bacteria (32%) in this study ($P<0.001$) and *E. coli* was the pathogen behind more than half of the infections. This is in accordance with results from several other studies, which reported the dominance of gram-negative bacteria and especially *E. coli* in their study populations **(12)**. The second most common gram-negative bacteria -*S. marcescens*- nevertheless, was not reported to be frequently isolated in UTIs in other studies, as normally, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Enterobacter*, *Citrobacter*, *P. mirabilis* are the most common bacterial uropathogens **(17)**. *S. marcescens* usually accounts for 1-2% of HAIs; however, its isolation rate was significantly higher in this study (5.38%) ($P<0.001$) **(18)**. This reveals that even though *S. marcescens* rarely causes community-acquired infections, its importance should be taken into account in healthcare settings and as a nosocomial infection, which plays a critical role in hospital outbreaks.

S. epidermidis and group D streptococci were the most prevalent gram-positive bacteria in the patient population with respective prevalence rates of 15.99% and 7.7%. This result was consistent with previous literature reporting *Enterococcus spp., Staphylococcus spp., and Streptococcus agalactiae* as dominant gram-positive uropathogens. Gram-positive bacteria nevertheless, are more likely to be contaminants in the urine cultures and should be analyzed along with other factors including symptoms and urine analysis results such as WBC count **(19, 20)**. *Lactobacilli*-containing samples were excluded from antibiotic susceptibility tests since they are a part of vaginal bacterial flora and commonly indicative of contamination while collecting urine specimens **(21)**.

Gram-negative bacteria were the most sensitive and resistant to nitrofurantoin and piperacillin with resistance rates of 25.27% and 76.04%, respectively. Gram-positive bacteria were widely resistant to ampicillin (68.61%) and Trimethoprim/Sulfamethoxazole (66.06%).

These results are in line with resistance patterns reported from Iran and worldwide **(3, 12, 17, 22, 23)**. Vancomycin resistance was relatively low in both gram-negative (26.74%) and gram-positive (19.34%) bacteria. However, it is considered as a last-resort antibiotic and should only be included in the antibiotic therapy regimen according to regional guidelines and only when needed. The vancomycin resistance result in our study was higher compared to that of a UTI study conducted by Mihankhah *et al.,* in northern Iran, in the same timeframe (11.7%) **(22)**. Furthermore, in a study by Khoshbakht *et al.*, conducted in Karaj, Iran in 2013, vancomycin resistance was reported 7.7% **(24)**. This rate was reported lower and under 5% in a ten-year surveillance European study conducted on the prevalence and susceptibility patterns of UTI-associated bacteria, in 2013 (13).

The limitations of the study included exclusive application of conventional biochemical microbiological tests for bacterial identification and not determining resistance-associated genes.

Conclusion

The overall prevalence of UTIs was relatively high in this study, which emphasizes the importance of proper quantity and quality of urine cultures and susceptibility tests, as well as development of novel efficient surveillance guidelines for each area. *E. coli* and *S. epidermidis* were the most prevalently isolated uropathogens and there was a notable *S. marcescens* outbreak in the study timeframe. Alarming resistance rates were observed in ampicillin and piperacillin, which should be taken into account in therapy guidelines in this area. Prevalence of resistant strains can be avoided by developing appropriate healthcare policies and community awareness. Further studies should focus on regional and periodical prevalence and susceptibility patterns of the UTIs, globally.

Limitations

Responsible genes to antibiotic resistance, genetic relationship between the resistant strains, and investigating any correlations with patient characteristics are not determined and these are the limitations of this study. Moreover, identification of isolates were merely performed taking advantage of biochemical aspect.

Consent for publication

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article/as supplementary information files.

Funding

Not applicable.

Authors' Contributions

Supervision, methodology, final editing and review were done by Bahman Mirzaei. Bahman Mirzaei and Erfan Fakheri contributed to Conceptualization. Narges Moradi and Niloufar Kazemi contributed to data collection, writing and data curation.

Acknowledgments

The authors are grateful for the support of colleagues in Bacteriology and virology Departments at Zanjan University of Medical Sciences.

Conflict of Interest

The authors declare that they have no competing interests.

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How to Cite This Article:

Fakheri E, Kazemi N, Moradi N, Mirzaei B. Bacterial Urinary Tract Infections in North West of Iran: A Cross-Sectional Study. J Adv Med Biomed Res. 2023; 31(138):464-71.