

# Prevalence and antibiotic resistance pattern of bacteria isolated from urinary tract infections in Northern Iran

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**Background:** This study aimed to investigate the bacteria associated with urinary tract infection (UTI) and antibiotic susceptibility pattern of the isolates during 2013–2015 in Northern Iran. **Materials and Methods:** Overall 3798 patients with clinical symptoms of UTI were subjected as samples, and they were cultured and pure isolated bacteria were identified using biochemical tests and subjected to antibiogram assessment using disc diffusion method. **Results:** Totally, 568 (14.96%) from 3798 patients had positive UTI. Four hundred and ninety-seven (87.5%) from 568 isolated bacteria were resistant to at least one antibiotic. *Escherichia coli*, *Staphylococcus* spp., and *Pseudomonas* spp. were the most prevalent bacteria. Isolated bacteria indicated the highest antibiotic resistance to methicillin (76.06%) and ampicillin (89.29%) and also revealed the most sensitivity to imipenem (99.1%) and amikacin (91.57%). Statistical analysis of the resistance pattern trend during 3 years indicated the insignificant increase ( $P > 0.05$ ) in antibiotic resistance of the isolates. **Conclusion:** The results of this study revealed a great concern for emerging UTI-related multidrug-resistant strains of bacteria causing UTI in Iran.

**Key words:** Antibiotic resistance, multidrug resistance, urinary tract infection

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## INTRODUCTION

Urinary tract infection (UTI) is one of the most common diseases in human societies which occur in women more than men.<sup>[1-3]</sup> The UTI occurrence depends on several factors provide the presence of bacteria (more than  $10^5$ /ml) in urine.<sup>[3]</sup> These bacteria cause UTI and if not treated, the infection will spread and cause serious damage to the patient.<sup>[2,4,5]</sup> UTI treatment with antibiotics is carried out usually before receiving microbiology test results. This therapy, without rational drug prescription occasionally leads to antibiotic resistance and treatment failure is its result.<sup>[3,6]</sup>

Discovery of antibiotics was one of the greatest advances of modern medicine,<sup>[7]</sup> but the availability and increased

use of antibiotics gradually lead to microbial resistance to them.<sup>[7]</sup> Antimicrobial resistance is increasing around the world, especially in developing countries.<sup>[8]</sup> According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a serious threat to modern medicine.<sup>[9]</sup>

The first important factor in increasing microbial resistance is improper use of antibiotics.<sup>[10,11]</sup> The other is incorrect and unreasonable antibiotics prescription. Considering time, the appropriate dose and manner of administration are the most important aspects of rational drug prescription.<sup>[11,12]</sup> Studies have shown that 30%–60% of the prescribing and use of antibiotics has been improper. Many hospitals have turned their supervision on the use of certain antimicrobial agents

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to change this worrying trend in Iran and all over the world.<sup>[13,14]</sup>

Although UTI is a common disease, it is treated easily if antibiotics are used reasonably.<sup>[2]</sup> Identification of bacteria that cause UTI and analysis of antibiotic susceptibility pattern of them is effective in the treatment.<sup>[15]</sup> The aim of this study was to investigate the bacteria associated with UTI cases and their antibiotic susceptibility pattern in the years 2013–2015 in Sari Avicenna Hospital, Northern Iran.

## MATERIALS AND METHODS

### Number of samples and sampling

This cross-sectional study was performed from March 2013 to 2015 in Sari, Iran Avicenna Hospital. Overall, 3798 patients with clinical symptoms of UTI were referred to the laboratory that 2137 (56.3%) patients were female and 1661 (43.7%) were male. Four ml of clean midstream urine of each patient were collected in a sterile tube and immediately transferred to the laboratory for investigation. Proper sampling instructions were given to each patient.<sup>[16,17]</sup>

### Standard colony count and identification of isolated bacteria

Ten microliter of urine were cultured on nutrient agar medium (NA; Merck, Germany) by cotton swabs in spread form and surveyed after incubation at 37°C for 24 h for colony count.<sup>[18]</sup> Positive UTIs consist at least 10<sup>5</sup> CFU/ml of bacteria in the urine. Results were repeated between 10<sup>3</sup> CFU/ml and 10<sup>5</sup> CFU/ml. Negative UTIs were <10<sup>3</sup> CFU/ml.<sup>[1]</sup> Then, colonies were cultured on a selective culture medium and examined by conventional biochemical tests to isolate bacterial agents of UTI.<sup>[19]</sup>

### Disc diffusion susceptibility testing method

In the current study, disc diffusion susceptibility test was performed on Mueller-Hinton agar (MHA; Merck, Germany) based on Clinical and Laboratory Standards Institute documents to determine the susceptibility of UTIs bacteria.<sup>[20]</sup> The antibiotic discs (PadtanTeb, Iran) were ampicillin (AM, 10 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), amikacin (AN, 30 µg), ceftazidime (CAZ, 30 µg), clindamycin (CC, 2 µg), ciprofloxacin (CP, 5 µg), ceftriaxone (CRO, 30 µg), nitrofurantoin (FM, 300 µg), gentamycin (GM, 10 µg), imipenem (IPM, 10 µg), methicillin (ME, 5 µg), nalidixic acid (NA, 30 µg), oxacillin (OX, 1 µg), penicillin G (P, 10 µg), co-trimoxazole (SXT, 1.25/23.75 µg), and vancomycin (V, 30 µg) that were available and routinely used in hospital.<sup>[21]</sup>

### Statistical analysis for antibiotic resistance trend of bacteria

Pearson's Chi-square test was performed to evaluate antibiotic resistance patterns of bacteria during 3 years.<sup>[22]</sup>

To evaluate the trend of antibiotic resistance, the proportion of resistant bacteria in the positive UTI was tested in these 3 years. A significant level of 0.05 was considered for this test.

## RESULTS

### Colony count and frequency of bacteria in positive urinary tract infection

In this study, 568 (14.96%) from 3798 patients had positive UTI result. Among them, 497 (87.5% of positive case) patients were resistant to at least one antibiotic. *Escherichia coli*, *Staphylococcus* spp., and *Pseudomonas* spp. were the most prevalent bacteria which were present in 188 (37.82%), 172 (34.6%), and 50 (10.06%) of the positive samples, respectively. The number of isolated bacteria by separating each year is presented in Table 1.

### Disc diffusion susceptibility testing

According to the results presented in Tables 2 and 3, among the isolated bacteria, the most resistant Gram-positive bacteria were *Staphylococcus* spp. (34.6% of the total) and the most resistant Gram-negative bacteria were *E. coli* (37.8% of the total). Gram-positive and Gram-negative bacteria showed the highest antibiotic resistance to methicillin and ampicillin, respectively, and also demonstrated the most sensitivity to imipenem and amikacin.

### The trend of bacterial resistance in 2013–2015

According to the results, the proportions of resistant bacteria in 2013, 2014, and 2015 were 84.9% (107 positive patients from 126 patients), 85.1% (160 positive patients from 188 patients) and 90.6% (230 positive patients from 254 patients),

**Table 1: Frequency of isolated bacteria from positive urinary tract infection patients in years 2013 (126 patients), 2014 (188 patients) and 2015 (254 patients)\***

Bacteria	2013			2014			2015			Total		
	R	N	%R	R	N	%R	R	N	%R	R	N	%R
<i>Escherichia coli</i>	42	9	82	52	13	80	94	15	86	188	37	84
<i>Staphylococcus</i> spp.	39	7	85	60	11	84	73	7	91	172	25	87
<i>Pseudomonas</i> spp.	8	2	80	19	3	86	23	1	96	50	6	89
<i>Klebsiella</i> spp.	5	1	83	19	1	95	15	1	94	39	3	93
<i>Enterobacter</i> spp.	5	0	100	4	0	100	6	0	100	15	0	100
<i>Proteus</i> spp.	4	0	100	1	0	100	4	0	100	9	0	100
<i>Acinetobacter</i> spp.	1	0	100	0	0	0	4	0	100	5	0	100
<i>Streptococcus</i> spp.	0	0	0	0	0	0	8	0	100	8	0	100
<i>Micrococcus</i> spp.	0	0	0	0	0	0	3	0	100	3	0	100
<i>Enterococcus</i> spp.	0	0	0	4	0	100	0	0	0	4	0	100
<i>Shigella</i> spp.	0	0	0	1	0	100	0	0	0	1	0	100
<i>Citrobacter</i> spp.	2	0	100	0	0	0	0	0	0	2	0	100
<i>Bacillus</i> spp.	1	0	100	0	0	0	0	0	0	1	0	100
Total	107	19	85	160	28	85	230	24	91	497	71	88

\*R = Resistant bacteria; N = Nonresistant bacteria; %R = Relative percentage of resistant to total

**Table 2: Resistant Gram-positive bacteria and the percentage of their antibiotic resistance**

Bacteria	Year	n*	n (%*)												
			AM	AMC	AN	CC	CP	CRO	GM	IPM	ME	OX	P	SXT	V
<i>Staphylococcus</i> spp.	2013	39	5 (13)	9 (23)	0	11 (28)	1 (3)	15 (38)	8 (20)	0	31 (80)	8 (20)	21 (54)	12 (31)	3 (8)
	2014	60	3 (5)	3 (5)	1 (2)	29 (48)	4 (7)	3 (5)	11 (18)	0	48 (80)	14 (23)	12 (20)	15 (25)	9 (15)
	2015	73	2 (3)	2 (3)	0	11 (15)	14 (19)	17 (23)	7 (10)	1 (1)	55 (75)	3 (4)	23 (32)	40 (55)	8 (11)
<i>Streptococcus</i> spp.	2015	8	1 (12)	0	1 (12)	0	2 (25)	1 (12)	2 (25)	1 (12)	3 (38)	0	3 (38)	5 (62)	2 (25)
<i>Micrococcus</i> spp.	2015	3	0	0	0	1 (33)	3 (100)	1 (33)	1 (33)	0	2 (67)	1 (33)	1 (33)	1 (33)	0
<i>Enterococcus</i> spp.	2014	4	0	0	0	2 (50)	0	1 (25)	1 (25)	0	3 (75)	2 (50)	1 (25)	1 (25)	0
<i>Bacillus</i> spp.	2013	1	0	1 (100)	0	0	0	0	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	0

\*n = Number of resistant gram-positive bacteria in each year; \*% = The percentage of antibiotic resistance, the breakdown of antibiotics, compared to resistant bacteria in each year. AM = Ampicillin; AMC = Amoxicillin-clavulanic acid; AN = Amikacin; CC = Clindamycin; CP = Ciprofloxacin; CRO = Ceftriaxone; GM = Gentamycin; IPM = Imipenem; ME = Methicillin; OX = Oxacillin; P = Penicillin G; SXT = Co-trimoxazole; V = Vancomycin

**Table 3: Resistant Gram-negative bacteria and the percentage of their antibiotic resistance**

Bacteria	Year	n*	n (%*)										
			AM	AMC	AN	CAZ	CP	CRO	FM	GM	IPM	NA	SXT
<i>Escherichia coli</i>	2013	42	39 (93)	18 (43)	2 (5)	8 (19)	6 (14)	10 (24)	7 (17)	16 (38)	7 (17)	13 (31)	14 (33)
	2014	52	42 (81)	2 (4)	1 (2)	5 (10)	7 (14)	2 (4)	6 (12)	18 (35)	7 (14)	23 (44)	27 (52)
	2015	94	85 (90)	1 (1)	1 (1)	17 (18)	19 (20)	28 (30)	7 (7)	37 (39)	17 (18)	38 (40)	43 (46)
<i>Pseudomonas</i> spp.	2013	8	6 (75)	7 (88)	0	0	1 (12)	3 (38)	4 (50)	8 (100)	1 (12)	1 (12)	3 (38)
	2014	19	16 (84)	2 (10)	3 (16)	8 (42)	6 (32)	9 (47)	8 (42)	18 (95)	4 (21)	7 (37)	15 (79)
	2015	23	22 (96)	0	3 (13)	13 (56)	4 (17)	14 (61)	13 (56)	18 (78)	3 (13)	12 (52)	18 (78)
<i>Klebsiella</i> spp.	2013	5	5 (100)	4 (80)	1 (20)	0	1 (20)	4 (80)	2 (40)	5 (100)	1 (20)	1 (20)	0
	2014	19	17 (90)	1 (5)	7 (37)	2 (10)	1 (5)	5 (26)	3 (16)	16 (84)	2 (10)	1 (5)	12 (63)
	2015	15	15 (100)	1 (7)	2 (13)	3 (20)	4 (27)	10 (67)	2 (13)	7 (47)	4 (27)	6 (40)	6 (40)
<i>Enterobacter</i> spp.	2013	5	5 (100)	2 (40)	3 (60)	0	1 (20)	4 (80)	1 (20)	5 (100)	2 (40)	1 (20)	3 (60)
	2014	4	4 (100)	0	1 (25)	0	1 (25)	3 (75)	0	1 (100)	0	2 (50)	3 (75)
	2015	6	6 (100)	0	0	1 (17)	0	2 (33)	1 (17)	2 (33)	0	0	1 (17)
<i>Proteus</i> spp.	2013	4	4 (100)	0	0	3 (75)	0	1 (25)	0	0	0	0	1 (100)
	2014	1	1 (100)	0	0	0	0	0	0	0	0	0	1 (100)
	2015	4	2 (50)	0	1 (25)	0	1 (25)	0	4 (100)	1 (25)	1 (25)	3 (75)	3 (75)
<i>Acinetobacter</i> spp.	2013	1	1 (100)	1 (100)	0	0	0	1 (100)	0	1 (100)	0	0	0
	2015	4	4 (100)	0	1 (25)	2 (50)	0	2 (50)	1 (25)	3 (75)	0	0	2 (50)
<i>Shigella</i> spp.	2014	1	0	0	0	0	0	1 (100)	0	1 (100)	0	1 (100)	0
<i>Citrobacter</i> spp.	2013	2	2 (100)	2 (100)	0	1 (50)	1 (50)	2 (100)	0	2 (100)	0	0	0

\*n = Number of resistant Gram-negative bacteria in each year; \*% = The percentage of antibiotic resistance, the breakdown of antibiotics, compared to resistant bacteria in each year. AM = Ampicillin; AMC = Amoxicillin-clavulanic acid; AN = Amikacin; CAZ = Ceftazidime; CP = Ciprofloxacin; CRO = Ceftriaxone; FM = Nitrofurantoin; GM = Gentamycin; IPM = Imipenem; NA = Nalidixic acid; SXT = Cotrimoxazole

respectively. To further investigation, the Pearson's Chi-square test was performed on these proportions with two degrees of freedom and significance level of 0.05. In this test, *P* value was obtained = 0.141 (significant or *P* > 0.05). In other words, the increase in bacterial resistance in each year is not significant. This amount represents a slight increase of bacterial resistance in 2015 compared to 2014 and also 2014 compared to 2013.

## DISCUSSION

The improper use of the antimicrobials for the treatment of the infections has adverse effects on public health organization of a country both in economic impact and increasing of the drug resistance among causative bacteria. Hence, it is essential to continuously evaluate the antimicrobial resistance condition in a society which

was the first purpose of the present study, particularly in the case of UTIs. At first glance, results of the study demonstrate relatively high occurrence of the positive urine culture among samples collected from patients with UTI clinical signs, in comparison with other studies in Iran.<sup>[23,24]</sup> It may be due to the climate and nature of the northern area of Iran, which has humid and relatively hot weather.<sup>[25,26]</sup>

As bacterial resistance increased in recent decades,<sup>[6,27]</sup> the isolates of the present study recovered from UTIs showed high resistance. Hence, 87.5% of them demonstrated resistance to at least one antibiotic. *E. coli* and *Staphylococcus*, similar to other studies were the most prevalent Gram-negative and Gram-positive bacteria, respectively.<sup>[23,28]</sup> The most resistance of the bacterial isolates was against methicillin and ampicillin, as higher than 75% of the *Staphylococcus* strains were methicillin-resistant *Staphylococcus* (MRS)

during 3 years. Similar results were obtained in other studies which not only show the increase of MRS *aureus* strains but also demonstrate the resistance of *Staphylococcus* spp. to other newer and alternative antibacterial agents such as linezolid.<sup>[23,29,30]</sup> Among Gram-negative bacteria, multidrug-resistant (MDR) strains have been reported as important and increasing strains which can spread the resistance among different populations of bacteria. *E. coli* and *Pseudomonas* spp. are the most significant Gram-negative MDRs, particularly in UTI patients.<sup>[23,31]</sup> One of the bacteria which represent pandrug resistance (PDR) in recent decade in Iran, particularly in hospital-related infections is *Acinetobacter* spp., which has become a critical issue in health-care system.<sup>[32]</sup> All five isolates of *Acinetobacter* recovered during 3 years from UTIs were PDR.

Results of antibiogram test for 497 bacterial isolates recovered from UTI revealed that amikacin and imipenem were the most effective antimicrobials against the strains. Some Gram-negative bacteria were resistant to these antibiotics, which are widely used for treating hospital-acquired infections with MDR Gram-negative bacteria such as *Pseudomonas* and *Acinetobacter*. Carbapenems are resistant to the  $\beta$ -lactamase enzymes produced by numerous MDR Gram-negative bacteria, so, playing a significant role in the treatment of infections not cured with other antibiotics.<sup>[33]</sup> Hence, probable increase of the imipenem-resistant strains can be an emerging concern for health control systems of a country. It seems that administrators should have a special precision and care in the use of these drugs for treatment of the UTI and/or other infections.

To survey the antimicrobial resistance pattern among patients with specific infection within a few years, it is essential to evaluate and compare antibiotic resistance condition in each of the years. The resistance pattern of 3 years (2013-2015) UTI-associated isolates recovered from Sari Avicenna Hospital, Northern Iran, were analyzed using statistical software. According to the results, there was an increase in the presence of the resistant bacteria among UTI isolates in Northern Iran from 2013 to 2015; however, this increase was not statistically significant ( $P > 0.05$ ). Immethodical and unprincipled antibiotic treatment and self-treatment by strong antibiotics cause such high levels of resistance among bacteria isolated from various infections, particularly UTIs. Nowadays, numerous organizations and programs are working to fight against antibiotic resistance<sup>[34]</sup> but first step to obtain a proper management and good control policy for decreasing the development of antibiotic resistance among microorganisms, particularly the pathogens is the evaluation and practical assessment of the antibiotic resistance patterns among definite populations of the patients of a country.

## CONCLUSION

The results of the present study revealed a slow increase of resistance among bacteria causing UTI in Iran and great concern for emerging UTI-related MDR strains.

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## Conflicts of interest

The authors have no conflicts of interest.

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