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CASE REPORT

Spectrum of Bacterial Resistance associated with Urinary Tract Infections from Clinical case in Northern of Iran

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ABSTRACT
Urinary tract infection (UTIs) is one of the most common bacterial infections that ar a big problems of health care in different countries. Thus, prevention of UTIs will lead to an overall decrease of antibiotic use. We aimed to evaluate the prevalence and antimicrobial resistance of bacteria caused in UTIs from clinical samples of Aliabd city of Gorgan province (Northern of Iran) during 2014. In sterilization manner, 5118 urinary samples were collected and cultured, and also the anti-microbial screening tests are done by disk diffusion method. After microbiological studies, 198 E. coli strains (47.14%), 129 Klebsiella spp (30.71%), 38 samples of Staphylocous epidermydis (9.05%), 55 samples staphylococcus saprophyticus (13.10%) have been verified from 398 positive sample cases. High resistance rates to Trimethoprim/ sulphamethoxazole (%28.28), Nalidixic acid and Trimethoprim/ sulphamethoxazole (36.43%), Nitrofurantoin (97.37%), and Trimethoprim/sulphamethoxazole (%32.73) respectively were documented. However, the most rate of susceptibility rates to Nitrofurantoin (44.94%), Gentamycin (%100), Nitrofurantoin (97.36%), and Trimethoprim/sulphamethoxazole (32.72%) respectively were recorded. The results of a study showed that the pattern of multiple antibiotic resistant, are observed perhaps is due to overusing this antibiotic in the study area. Future monitoring studies to improve the diagnostic criteria is recommended.
Keywords *Urinary tract infection, Antibiotic resistance, Antibiotic*

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INTRODUCTION

After respiratory tract infection, the second most common bacterial infections are Urinary tract infections (UTIs) that the highest degree of importance in the human's lifespan [1-2]. UTIs is kind of infections are considered as the presence of microbial pathogen in urinary tract [1-2], and the frequency in women is more than men, but also, its frequency, symptoms and causative organisms diverse in accordance with sex, age, and gender and miscuing might result in disordering urinary tract (UT), blood pressure and prematurity. Verifying bacterial agents and using efficient antibiotic is a practical solution for eliminating infection and prevent its consequences [3]. The most commonly pathogenic microorganisms in urinary tract responsible for UTIs are *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus faecalis* [4]. According to statistical literature, about 2% of hospitalized patients will suffer from UTIs during the time of presence of them in a hospital and overall about 900000 infections of urinary is made in a hospital [5]. Studies in several societies show that Gram-negative

Bacillus bacteria is the most common factor of UTIs and among them, *E. coli* that are found in stool makes more than 80% of acute UTIs [6], and about 75% of urinary infection in men is caused by *E.coli* [1]. Other microorganisms which cause infection such as, *Proteos mirabilis*, some of *Klebsiella*, *Entrobacter,* and *Puesodomons*. Increased risk of UTIs reported in infants, pregnant women, oldest, the patients using urinary sond continuously, diabetics, MS, HIV and medullar patients [7]. Bases of appropriate treating UTIs are choosing an efficient antibiotic, but the main problem, is the presence and development of resistance strains to antibiotic treatment that resulted in increased incidence of treatment failure. The worldwide emergence of multidrug-resistant pathogenic microbe is mostly due to the genetic characteristics of bacteria, increasing in population, traveling and overusing antibiotic [8]. The choice of antimicrobial agents is more challenged and should be individualized based on the patient's allergy history, local practice patterns, the prevalence of resistance, availability, cost, and compliance [9]. In this study, aimed to advances in antimicrobial therapy, the frequency of bacterial pathogenic agents in urinary tract infections and antibiotic susceptibility pattern of them were determined in the hygienic clinical center in Aliabd city of Gorgan province (Northern of Iran) during 2014.

MATERIALS AND METHODS

In the present study, 5118 urinary samples were collected. After diagnosing peoples' age and gender, urinary samples were gathered using midsestream clean catch by standard techniques [10]. Urine samples, by a standard loop, has been cultured in sterilization plate of environment Sheep Blood agar (SBA), and Eosin Methylene Blue (EMB) agar and then incubated at 37^0 C for 24 h. As the standard operation procedures, a colony was counted and those samples that numbers of grown colonies were equal or more than 10^5 per millimeter have been considered as positive (colony forming unit (CFU/ml)) in view of UTIs. Next, the different organisms were identified by standard biological and biochemical tests (API strips; bioMérieux) and quantified. According to CLSI advice, a colony of bacteria from the overnight culture was determined for antibiogram test by disk diffusion method on Muller Hinton Agar medium against different antibiotics [11]. Diameters of any resulting zones of inhibition (mm) of growth were then measured and reported in millimeters based on CLSI advice. The frequency of susceptibility and resistance against bacteria caused infection in relation to antibiotics such as, Tetracyclin (TE), Ciprofloxacin (CRO), Amikacin (AN), Ampicillin (AM), Gentamycin (GM), Nitrofurantoin (FM), Trimethoprim Sulfamethoxazole (SXT), Nalidixic Acid (NA), Clindamycin (CN), Ceftriaxone (CP), Norfloxacin (NOR), prepared by Padtan Teb company was studied. The SPSS 19.0 (IBM, SPSS) software package for Windows was used to analyze of a variance of the raw data. All data are reported as mean ± SD and by using Duncan's multiple range tests in ANOVA, significant differences between means were identified. The chi-square test was used to compare the data. A statistically significant bacterium was considered if P value of < 0.05

RESULT AND DISCUSSION

During 2014, a total of 5118 UTIs samples, were collected and analyzed for isolation of bacteria and operational procedures antimicrobial susceptibility test. As a view of sex, a number of 4771 cases (93.23%), 314 cases (6.13%) and 32 cases (0.62 %), were women, men, and children respectively. Among 4771 samples of women, 1479 cases (30.99%) and 3274 cases (68.62 %) were pregnant and nonpregnant respectively. As a view of age, the data illustrated the age distribution of patients was based on decade showed the range between in the most number, 21-30 and the least number 0-10, years old. After microbiological survey, 198 samples of *E. coli* (47.14%), 129 samples of *Klebsiall spp* (30.71%), 38 samples of *Staphylococcus epidermidis* (9.05%), and 55 samples of *Staphylococcus Saprophytics* (13.10%) (Fig 1), were screened from 398 positive sample cases of women (7.8%), 10 cases men (0.19 %), 12 cases children (0.23 %) (Table 1).

Table 1: The frequency of experimental bacteria strains from UTIs in men, women, and children

Total	*S. Saprophytics*	*S. epidermydis*	*Klebsiella spp*	*E.coli*	
10	0	0	2	8	Male
2.38%	0	0	20%	80%	
398	55	38	124	181	Female
94.76%	13.82%	9.55%	31.15%	45.47%	
12	0	0	3	9	children
2.86%	0	0	25%	75%	
420	55	38	129	198	Total
100%	13.10%	9.05%	30.71%	47.14%	

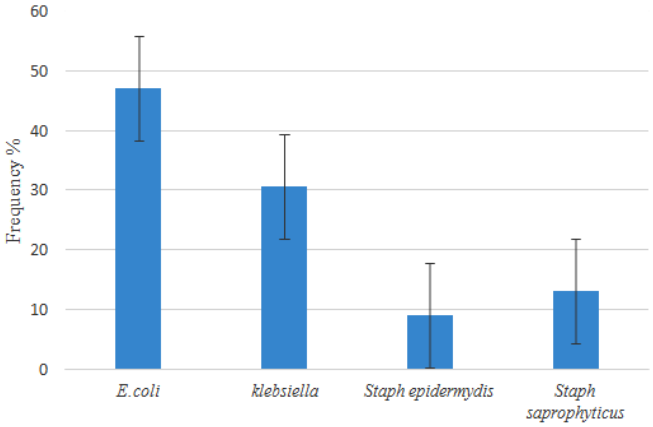

Figure 1*:* Distribution of relative frequency of obtained bacteria from UTIs patients based on age

The results of antibiogram test showed that the bacteria such as *E. coli*, *Klebsiall spp*, *S. epidermidis*, and *S. Saprophytics* have the most rates of susceptibility against, Nitrofurantoin (44.94%), Gentemycin (100%), Nitrofurantoin (97.36%), and Trimethoprim/sulphamethoxazole (32.72%) respectively (Fig. 2-5). The highest resistance rate against bacteria (*E. coli*, *Klebsiall spp*, *S. epidermidis*, and *S. Saprophytics*) were documented to Trimethoprim/sulphamethoxazole (28.28%), Nalidicxic acid and Trimethoprim /sulphamethoxazole (36.43%), Nitrofurantoin (97.37%), and Trimethoprim/ sulphamethoxazole (32.73%) respectively (Table 2). As shown in table 4, the overall multiple drug resistance rate in four isolated bacteria showed the decrease trend (<50%) to 11 antimicrobials tested, with the exception of only (97.37%) to Nitrofurantoin in *S. epidermidis* strains. As shown the chi-square test in table 3, finding indicated the risk factors for the emergence of antibiotics resistance bacteria prior antibiotic therapy were significant.

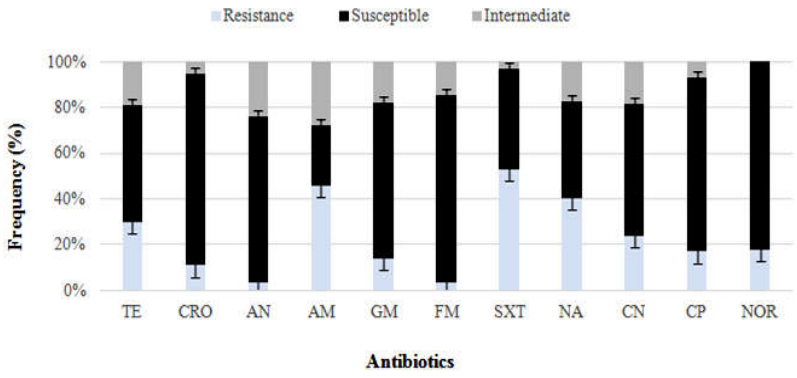

Figure 2: Antibiogram results in *E. coli*

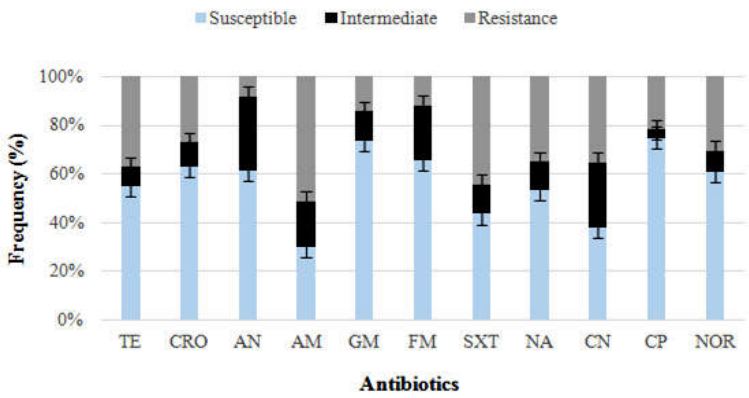

Figure 3: Antibiogram results in *Klebsiella spp*

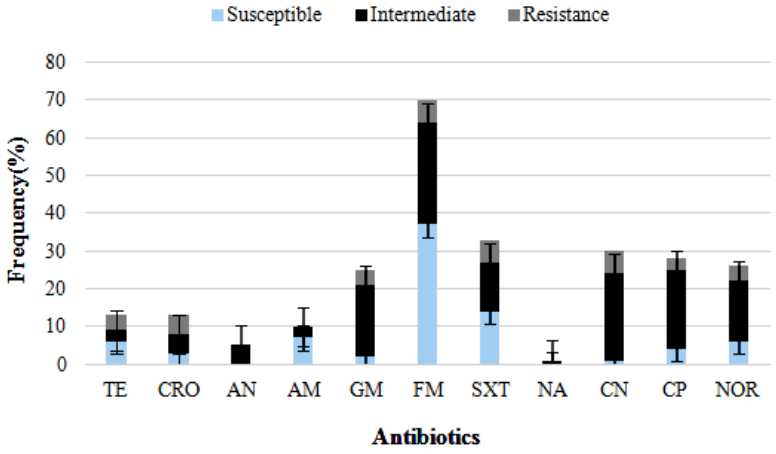

Figure 4: Antibiogram results in staphylococcus epidermydis

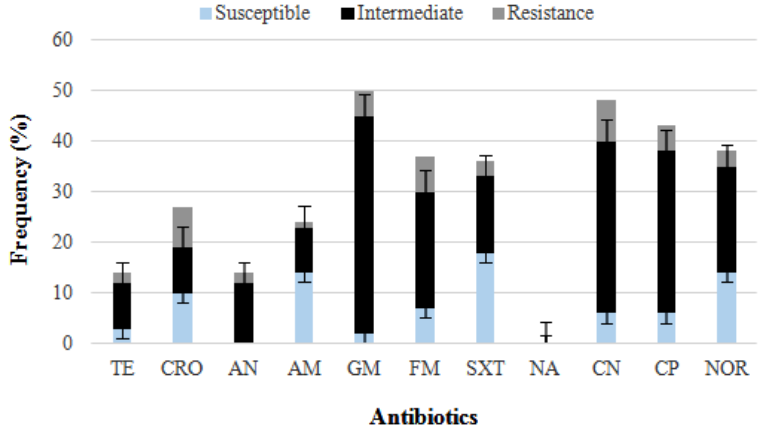

Figure 5: Antibiogram results in *S. saprophyticus*

Table 2: The frequency of resistance of Antibiotic pattern of separated strains bacteria from UT

Antibiotics	*E. coli* N=198	*Klebsilla spp* N=129	*S. epidermydis* N=38	*S. saprophyticus* N=55
TE	29(%14.65)	14(%10.85)	6(%15.79)	3(%5.45)
CRO	39(%19.71)	6(%4.65)	3(%7.9)	10(%18.18)
AN	7(%3.54)	1(%0.77)	0(%0.00)	0(%0.00)
AM	41(%20.71)	23(%17.83)	7(%18.42)	14(%25.45)
GM	25(%12.63)	17(%13.18)	2(%5.26)	2(%3.64)
FM	15(%7.58)	4(%3.10)	37(%97.37)	7(%12.73)
SXT	56(%28.28)	47(%36.43)	14(%36.84)	18(%32.73)
NA	42(%21.21)	47(%36.43)	0(%0.00)	0(%0.00)
CN	48(%24.24)	23(%17.83)	1(%2.63)	6(%10.91)
CP	27(%13.64)	17(%13.18)	4(%10.53)	6(%10.91)
NOR	22(%11.11)	3(%2.33)	6(%15.79)	14(%25.45)

Table 3: Risk factors with respect to the occurrence of UTIs due to antibiotics resistance bacteria

Risk factors	Chi-squre (X^2)	Odd ratio (%95 CI)	Relative risk (%95 CI)
Females vs male	P=0.0018	2.7668 (1.4615 to 5.2379)	2.6194 (1.4132 to 4.8550)
Adults vs children	P<0.0001	0.1454 (0.0706 to 0.2995)	0.1284 (0.0966 to 0.1706)
Male vs children	P<0.0001	0.0548 (0.0211 to 0.1422)	0.0849 (0.0399 to 0.1809)
Female vs children	P<0.0001	0.1517 (0.0736 to 0.3126)	0.2225 (0.1408 to 0.3514)

Urinary tract infection with frequency about 250 million patient per year is one of the big problems of health organizations in different countries [12]. Mandal *et al* (2012) showed that *E. coli* were the commonest pathogen causing complicated and uncomplicated UTIs [13]. This bacteria is one of the important pathogenic microorganisms that show an increase of resistance in relation to the most of the antibiotic [14-15]. According to the importance of UTIs, this study has been done in the hygienic clinical center of Northern of Iran. Here the numbers of suffering women (93.23%) from urinary tract infection are more than men and children (6.73%) that probably its reason is shortness of urine's way and being close to vagina and rectum in women. The most of the UTIs was observed at ages 21-30 and after that is 31-40. The results showed that bacteria of Enterobacteriaceae are the most common agent of UTIs and among them, *E. coli* is pathogen generating UTIs with the most frequency (47.14%) and *Klebsiella spp*, is the next one with isolation rate is 30.71%, that conformity with reports of other studies[15-16], and after that *S. saprophyticus* and *S. epidermydis.* The result showed the highest resistance rate against bacteria (*E. coli*, *Klebsiall spp*, *S. epidermidis*, and *S. Saprophytics*) were documented to Trimethoprim/ sulphamethoxazole (28.28%), Nalidixic acid and Trimethoprim/sulphamethoxazole (36.43%), Nitrofurantoin (97.37%), and Trimethoprim/ sulphamethoxazole (32.73%) respectively. On the other hand, the overall resistance of all studied bacteria to antimicrobials test at the present study based on table 4 was nearly low, especially in *E. coli*, but among them, *S. epidermidis* showed high resistance rates of >90% to Nitrofurantoin and 36.84% to Trimethoprim/ sulphamethoxazole. The overall multiple drug resistance rate in four isolated bacteria showed the moderate trend (<50%) to 11 antimicrobials tested, with the exception of only (97.37%) to Nitrofurantoin in *S. epidermidis* strains. So, it seems the rate of bacterial resistance observed in the study area is not still worrying. According to the clinical resistance rate results, in earlier treatment of UTIs would be better some of the antibiotics, such as Trimethoprim/ sulphamethoxazole and Nitrofurantoin are less used. In agreement with the result, Vaezzadeh and Sharifi-Yazdi [17] demonstrated that the *E. coli* was the most frequent etiologic agent (75.62%) followed by *klebsiella* species (7.32%), and the antimicrobial resistance rate of *E. coli* was recorded to routinely antibiotics: cotrimoxazole (82%) and ampicillin (82%), but none of them were resistant to ceftizoxime. Alizadeh Taheri et al [10] also showed the *E. coli* was the dominant (64.4%) and mostly resistant to ampicillin (93.6%), cefixime (85.7%) and cephalexin (77.3%), and sensitive to cefotaxime (63.6%), another bacterium such as, Enterobacter (19.2%), Klebsiella (12.3%), and *S. epidermdisis* (4.1%) were less frequent isolated bacteria. But between them, Enterobacter found to be most resistant to amikacin (100%), ampicillin (92.85%), and most sensitive to ceftizoxime (71.4%). Resistance species of these bacteria are increasing in substantial geographic variation as well as differences in population and environment [18], and the problem started as patients do not complete the treating period and a live bacterium gets resisting. In many cases, we faced drug resistance in pathogens due to overusing antibiotic that itself may be the crucial reason of unsuccessful treating and appearance of complications despite spending the high costs of health. In this study, Nitrofurantoin, Gentamycin, and Trimethoprim/ sulphamethoxazole were found to be the most effective antimicrobials against studied bacteria. Similar studies conducted in many other plases but the resistance rates recorded in this study are lower than the results of Kibret and Abera [15] and in agreement with the result of Kashef et al. [19]; Khameneh and Afshar [20]; and Kothari and Sagar [21], findings of the present study show that *E. coli* is the predominant and resistance pathogen of UTIs. Resistance drugs in relation to the antibiotic in various regions of Iran and world due to genetic changes in producer equality and different in the rate of using antibiotic and differences in access to the antibiotic. The resistance of bacteria against antibiotic is inherent and acquired. In the inherent resistance (Chromosomal or Plasmids), the cell inherited alleles that are the agents to prevention the effect or antibiotic operation and creating the resistance equality among susceptible of bacteria are presented in antibiotic exposure [22]. Beyene and Tsegaye [23] also reported that *E. coli* isolates were the predominant pathogens with very high resistance to the commonly prescribed drugs that in turn leaves the clinicians with very few alternative options for drugs for the treatment of UTIs. Their showed that 100% of *E. coli* and *K. Pneumoniae* isolates were resistant to Amoxicillin and Ampicillin. So, this finding reported these antibiotics cannot be used as empirical therapy for urinary tract infection particularly in the study area. But in present study both of *E. coli* and *Klebsiella spp*, isolates to some extent were resistant to 11antibiotics. However, based to increase the resistance against antibiotics, quick and on time diagnosing of resistance equality in order to choose suitable treating options and preventing from resistance distrobution is necessary. Hence continuous evaluation of bacteriology and true treating to diagnosis of symptomatic UTIs is necessary to the usage of antibiotic disks in labs, and to prevent of resistance to new drugs from the use of improper and irregular and failure in treatment that leading to complicated infection. In conclusion, The results of this paper show that pattern of multiple antibiotic resistants in Northern of Iran (Aliabd city of Gorgan province) are exists in

different bacteria such as *E. coli*, *Klebsiall spp*, *S. epidermidis*, and *S. Saprophytics,* caused in UTIs. The highest rates of antimicrobial resistance were recorded in *S. epidermidis* to Nitrofurantoin. Nitrofurantoin, Gentemycin, and Trimethoprim/ sulphamethoxazole are considered appropriate for empirical treatment of *E. coli*, *Klebsiall spp*, *S. epidermidis*, and *S. Saprophytics* respectively. However, future monitoring studies to improve the diagnostic criteria for UTI in adults, particularly those living in long-term care facilities, are needed.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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