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ORIGINAL ARTICLE

Surveillance of Multidrug Resistant Uropathogenic Bacteria in Hospitalized Patients in Iran-Tehran

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ABSTRACT

Urinary tract infection is second widespread infection in human body and regarding its bad consequences prescribing appropriate antibiotic and at suitable time is very important. What in recent years has made medical community highly concerned is resistance against antibiotics. With increase of drug resistance among microorganism some antibiotics that were effective at a certain moment, currently have very low effect on bacteria causing urinary tract infection. This event takes place for the reason of nonstandard usage of antibiotics. The objective of present study is to examine antibiotic resistance and determining prevalence of multidrug resistance in isolated microorganism from urinary tract infections in Iran. Within one year from total of 3635 urinary sample from patients hospitalized in three hospitals of Iran number of 420 cases reported positive culture. Among isolated pathogens Escherichia coli with 227 cases (54.04 %) reported as most widespread isolated organism. Highest drug resistance of Escherichia coli is reported related to Ampicillin 80.7% cotrimoxazole 37% and cefalotin27.7%. From one side most sensitivity was related to antibiotics such as norfloxacin 89.6%, nitrofurantoin 89%, and ceftizoxime77.3%.
Key words: multidrug resistant, uropathogenic bacteria, UTI, MDR, Hospitalized patients, Iran.

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INTRODUCTION

Urinary tract infection (UTI) is one of the most widespread infections of all age groups that failure in on time diagnostic and its treatment can brings about severe consequences in patient. Near 150 million persons per year suffer from UTI[1]. Infectious factors causing UTI are very versatile and include fungous and virus factors but bacterial infections are dominant cause of these infection and bring about 95% of hospitalurinary tract infections. Conducted investigations in various societies shows that mostly etiologic factors of UTI belong to bacteria of family enterobacteria among which *Klebsiella* pneumonia and Escherichia are considered ad most important factors for prevalence of urinary tract infections.

Among urinary pathogens the genus Escherichia coli (57.9-72.7 %), klebsiella (2.7-9.88 %) proteus (5.6-7.1%) pseudomonas (2.9-6.52%) streptococcus (2.68-17.8%) staphylococcus (0.5-6.35 %) are the most prominent bacteria causing urinary tract infections [2-5]. The ability of bacteria in attaching to urinary tracts is an important feature in its pathogens. Many types of E.Coli enjoy from such ability. Other ability of E.Coli that helps to its pathogenesis includes high resistance against antibacterial property of human serum, producing hemolysis and increasing production of Capsule antigen K , existence of pilus on cells

surface of urinary tract, promotes the possibility of E.Coli to attach to tract cells and foster the probability of UTI appearance[6, 7].

This is more than fifty years that antibiotics are used for rapid and effective treatment of infectious diseases. Within this period many changes have been taken place in types of used antibiotics as well as sensitivity and resistance of bacteria against them [8].within recent years resistance level of antibiotics has been increased that benefits from geographic diversity as well. Also recently in addition to general discussion of antibiotic resistances, bacteria drug resistance to two types of different antibiotic is called multidrug resistance (MDR) has been increased particularly within hospital isolations in such manner that emerged infections by negative warm basils with multidrug resistance is identified as one of the important causes of death among hospitalized patients [9]. Bacterial resistance can be as internal resistance (lack of natural sensitivity of all organs of a genus of bacteria to antibacterial drug) or acquired resistance and lack of sensitiveness of some certain strains of a genus of bacteria to antimicrobial drug [10-12]. Resistant strains are dramatically increasing due to easy conditions for transference in different physical and chemical conditions in hospitals throughout of world. Many microorganisms have become resistant to various antibiotics and sum of them to all antibiotics and this leads to death increase especially among patient with immune system deficiency. In developing countries there is limited information about hospital infection prevalence. Infections prevalence are approximated near 65% in some centers [13].

The studies showed that regardless to antibiotics usage pattern antibiotic resistance genes can transfer between bacteria population [14]. Antibiotic resistance genes usually exist in genetic elements called integrons[11, 12, 14]. Integrons are considered as new mechanism for resistance genes spread among bacteria[15, 16]

In Iran drug resistance is increasing in hospitals especially in special wards [17]. Acquired resistance can be due to bacterial chromosome mutation, in such cases change in specific genetic position can cause resistance in bacteria, this leads in reducing sensitivity to antibiotic and at the same time vital actions of cell would continue. Producing drug resistance rarely takes place through genetic mutation. Among other ways of drug resistance is obtaining new chain from DNA that can transfer between bacteria or translate constantly from generation to another. The new resistance gene can enter the bacteria chromosome or can be out of chromosome (Plasma). Sometime a plasmid obtained from drug resistance can be disappeared by taking desired antibiotic [18, 19].From one side producing enzymes of beta-lactamase leads in microorganism resistance against penicillin, cephalosporin and monobactam. Beta-lactamase enzymes family are highly heterogenic. Plasmids containing these enzymes often carry the resistance gene to other antibiotics as well. Therefore microorganisms containing these genes have pivotal role in antibiotic resistance transference that awareness from forms of regional resistance of Escherichia coli bacteria is the main condition of treatment.

Thus nowadays one of the most important problems in treating infectious diseases is bacterial resistance of pathogens to antibiotics. Among pathogens of urinary system especially Escherichia coli resistance can be taken into account that is resistance to ampicillin 18-54 % and 9-27 % to trimethoprim and 16-49 % to sulfamethoxazole. The resistance to nitrofurantoin and fluoroquinolones are less (near 3 %).

In recent years various studies have been conducted in different parts of the world on the antibiotic resistance in microorganisms. The result of these studies as total reveals increase of Escherichia coli frequency resistant to multidrug. In investigation of American researchers the resistance tomultidrug in Escherichia coli is declared 7.1%. Moro et al (2014) have studied diversity and antibiotic resistance of uropathogenc bacteria from Abidjan. For this purpose 12175 urine sample have been collected within a 12 years period (2000-2011) from Treichville teaching hospital according to prevalent protocols. The result for obtaining prevalence of specifications of UTI was successful. The rate of bacterial resistance to antibiotics depends on their evolution level within the time and multidrug resistance level [20].

Some studies have been conducted in Iran too. Moniri et al (2012) have studied emergence of multidrug resistant strains of Escherichia coli isolated from urinary tract infections. The study has been carried outfrom April to November 2001 they evaluated the incidence of resistance to trimethoprim-sulfamethoxazole (ZXT), gentamicin, cephalothin, ciprofloxacin, and nitrofurantoin in 220 Escherichia coli isolated from patients with urinary tract infections in Kashan, Iran. For evaluating the current spread of multidrug resistance among urinary isolates of E. coli. Of total isolated, 10.9% were resistant to three or more agents and regarded as multidrug resistant. Among the multidrug resistant isolations, 91.7% have been resistant to SXT, 75% to gentamicin, 58.3% to cephalothin, 54.2% to ciprofloxacin and 45.8% tonitrofurantoin. The main make-up between multidrug resistant isolations(29.2 %) included resistance to SXT, gentamicin,and cephalothin, rates of multidrug resistance have been revealed to be higher in males (13.2%) than females (10.4%). There was no significant relation between gender and reduced

susceptibility. Continued local monitoring studies are necessary to monitor emerging antimicrobial resistance and to guide measures and practices to minimize its emergence[21].

The basis of appropriate treatment in urinary tract infections is selecting high efficiency and cheap antibiotics. Regarding ever-increasing antibiotic usage and in consequence increase of antibiotic resistance as well as diversity in sensitivity of E. Coli in different parts of the world, examining antibiotic resistances of urinary pathogens such as E. Coli seems to be indispensable.

METHODOLOGY

Within a one year from November 2013 to November 2014 number of 3435 urine samples have been collected from hospitalized patient from urologic ward in Labafinejad , Taleghani and Shohada hospital of Iran , Tehran that have clinical symptoms of UTI and have been cultures on blood agar and McConkey agar environment. The samples that their colony number was more than 100000 in each mm was considered positive and then the genus and type of each bacterium was identified based upon standard methods. In this investigation antibiotic sensitivity of isolated bacteria was determined by the method of disc diffusion in agar according to instruction of CLSI to 14 antibiotic disk including ampicillin (10 mg), norfloxacin(10 mg) cefalotin (30 mg) ciprofloxacin (5 mg), gentamicin (10 mg), amoxicillin (10 mg), nalidixic acid (30 mg), nitrofurantoin (300 mg), tetracycline (30 mg) and cotrimoxazole (25 ml). The result of antribiogram of each one of samples was read and interpreted after 24 hour of incubation in temperature of 37 degree centigrade according to CLSI table. In this stage the isolated resistant to at least three antibiotics from different families are selected as MDR strains. Sensitivity determination test is carried out on the standard strain of E. coli ATCC 25922 exactly in the same manner and the results would be compared with standard values. After 18-24 hours of incubation of plates containing bacteria in temperature 37 degree centigrade the results are examined and the region of obstructing growth is measured by ruler in terms of millimeter and obtained results are registered in the form of sensitive, resistant and average. Finally obtained data from study are examined and analyzed by statistical test of Chi-square and SPSS software.

RESULTS

Number of 3635 urine sample are collected from hospitalized patients from Taleghani, Labafinejad and Shohada hospitals of Tehran (Iran) within a period of 12 months (November 2013 to November 2014). Among conducted experiments 1905 samples belonged to males and 1730 sample for females. Among cultured samples number of 420 positive cultures has been reported. Among which number of 345 cases belonged to female and 75 cases to males. As a result it was revealed that UTI prevalence in women was more than men. Among isolated pathogens Escherichia coli with 227 cases (54.04%) was reported as the most prevalent isolated organism (in significance level P=0.000) among which 167 cases was for women and 60 cases for men (table 1).

Most drug resistance of Escherichia coli was associated to antibiotic ampicillin 80.7%,cotrimoxazole 37% and cephalothin 27.7 %. From other side most sensitivity was associated to antibiotic norfloxacin 89.6%%, nitrofurantoin 89% and ceftizoxime 77.3%. (Table 2).

Table 1- types of isolated microorganisms

Bacteria type	number	percentage
E. coli	227	54.04
Coagulase negative staphylococci	41	9.76
Streptocarpus sp.	24	5.71
Enterococcus	13	3.09
Citrobactersp.	12	2.85
Klebsiella sp.	91	21.66
yeast	2	0.47
Total	420	100

Table 2- frequency distribution of antibiotic resistance of Escherichia isolated from urinary infections

Antibiotic	Frequency and sensitive cases percentage	Frequency and relatively resistance cases percentage	Frequency and resistance cases percentage
Ampicillin	22 (8%)	31 (11.3%)	174 (80.7%)
Cotrimoxazole	141 (62%)	3(1.5%)	83 (37%)
Cefalotin	131 (58.1%)	33 (14.5%)	63 (27.7%)
Gentamicin	174 (76.8%)	5 (1.9%)	48 (21.3%)

Amoxicillin	118(52%)	51(22.4%)	58(25.6%)
Tetracycline	175(77.3%)	18(8.1%)	33(14.5%)
Imipenem	132(58.3%)	88(38.6%)	7(3.1%)
Ciprofloxacin	110(48.6%)	71(31.2%)	46(20.2%)
Amikasin	83(36.4%)	84(37.3%)	59(26.3%)
Nalidixic acid	93(41.2%)	66(28.5%)	68(30.3%)
Chloramphenicol	99(43.7%)	64(28.2%)	64(28.1%)
Norfloxacin	202(89.6%)	12(5.5%)	13(5.5%)
nitrofurantoin	202(89%)	18(8%)	7(3%)
ceftizoxime	175(77.3%)	43(18.7%)	9(4%)

DISCUSSION

Level of antibiotic resistance between isolated obtained from society and hospital is increasing. This affair is an immense and worldwide problem. Resistance in strains causing urinary tract infection which is one of the most prevalent infection is increased and worrying issue [22]. This can be attributed to irrational and ever-increasing use of drugs. Unfortunately Iran is not accepted from this rule and prescribing a lot of drugs by medicines and incorrect usage of antibiotics by patients leads to prevalence of resistant strains in the society.

In a study conducted by Matti et al (2004) on Escherichia coli and klebsiella causing urinary tract infection have demonstrated the most level of antibiotic resistance is to ampicillin, cotrimoxazole and tetracycline[23]. In another study taken place in 2006 by Rijavec most level of resistance was to ampicillin, tetracycline and chloramphenicol[24]. In another study it was revealed that E. coli with 61.41 % is most prevalent factor causing urinary tract infection. Also most level of resistance of factors producing urinary tracts infection regarding bacteria strains was observed to cefixim (84.5%) and sulfamethoxazole (66.14%)[25]. In conducted studies in other parts of Iran and world this organism was identified as most prevalent factor causing urinary tracts infections, and its prevalence is reported at 75.58% to 45.4%. in study of Kader et al on 11659 sample in Saudi Arabia 58% [26] and in the study of Akram et al on 920 samples in India 60% [27] and in study of Rostamzadeh et al on 803 samples in Iran 78.87% [28].

Borsari et al (2008) have reporter most level of Escherichia coli strains resistance in urine samples of children to ampicillin and cotrimoxazole and least resistant to nitrofurantoin [29].

Temberker et al in 2006 in India after examining 68 urinary samples achieved to isolation of 59% of E.Coli strains. In their study most level of resistance was reported to ampicillin (87%) and cotrimoxazole (91%) and least level to nitrofurantoin (29%) [30].obtained result of Tankhiwale et al study in 2004 has demonstrated that most level of resistance of E.Coli strains was to cotrimoxazole (82%) and ampicillin (79.9%) and least resistance to nitrofurantoin (38%) [31]. Andrade et al in 2006 in a study examined 611 urinary samples. E.Coli strains were isolated in 66% of cases. Also most level of resistances in strains was to antibiotic ampicillin 53.6% and leas resistant was to imipenem (0%) [32].

Cotrimoxazole is an antibiotic with wide spectrum that has abundant applications in urinary tract infection and etc. however in the present study 37% of strains were resistant to this antibiotic. This can suggest the ever-increasing of pathogen bacteria resistance to cotrimoxazole. Cefalotin belongs to first generation antibiotics of cephalosporin that has been used as first line drug for treatment of a lot of urinary tract infection. But in current study regarding resistance of 27.7% of strains significant resistance to antibiotics is observed and this can be attributed to irrational use of this group of drugs.

Since the antibiotic nitrofurantoin is among drugs selected for treatment of urinary tract infection and also regarding strains significant sensitivity (89%) to this antibiotic in case of emergence of drug tolerance by the patient this antibiotic can be used as an appropriate drug for treatment of urinary tract infection. In current study the amount of sensitivity to the antibiotic ceftizoxime was 77.3%. Therefore there are still hopes that prevalence level of drug prevalence to third generation of cephalosporinis not prevalent among isolated microorganism. Maybe it's due to the low usage of this group of drugs for treatment of urinary tract infection.

In this study it has been revealed that microorganism percentage resistance to multidrug is highly significant. With regard to the fact that treatment of strains that have become resistant to several antibiotic is highly difficult, especially in individuals with background disease or weakness of immune system. Therefore spread of these strains in society or in hospital infections is quite important. Thus necessary measures must be seriously taken into account for infection control as well as observing sanitary regulation in order to eliminate or decrease this kind of strains especially in hospitals.

From other hand the prevalence level of urinary infection among women is higher than men. These findings are consistent with researches carried out around the world [27, 33-35]. The reason of these results can be reckoned as the difference of anatomy and physiology between men and women [36, 37]. The observed difference in obtained responses by other studies can be argued that most researches have taken place in European countries with lower antibiotic consumption and as a result the resistance level has been lower. From other hand, strain distribution resistant to drug varies in different geographic regions and depends on consumption pattern. Also the number of examined strained in mentioned articles was very less than the current study and just the antibiotics such as quinolone group have been evaluated. The type of the examined sample can be another reason of these differences.

CONCLUSION
Based on this study E.Coli has been identified as the most prevalent cause of urinary tract infection. In the studied region most level of resistance in both genders regardless to bacteria strain have been to ampicillin and cotrimoxazole and cefalotin and these two drugs are not recommended as the first line antibiotic treatment. Also most level of sensitivity has been to norfloxacin that could be recommended for antibiotic first line treatment. Antibiotic resistance pattern varies within different regions and the resistance to more recent antibiotics is increasing too. Thus it seems that analysis of different microorganism isolated from urinary infections and other extra intestinal infections especially in different geographical regions can offer useful information in the area of phylogenetic groups' epidemiology and strain distribution resistant to drug. In a manner that with relying on them and by help of improving treatment methods and necessary education about correct usage of antibiotic the spread of strains resistant to antibiotics in the society can be obstructed. Thus it is recommended that examination of antibiotic resistance pattern takes place periodically and within few years for using in empirical and specialized treatment of urinary tract infections.

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