Research Article

Investigating the frequency of multi-drug resistant strains of escherichia coli isolated from urinary tract infection in children

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Abstract

Introduction:
A current concern in the medical community is the rise in multi-drug resistant (MDR) organisms and their respective problems for children in developing countries. Children infected with such organisms need more care, hospitalization and expensive drugs for their therapy. So, an attempt was made to evaluate the multidrug resistant strains of *Escherichia coli* isolated from urinary tract infection in children from Jahrom to recognize the factors involved and control the use of antimicrobials in this area to select best strategies.

Material and Methods:
This cross-sectional descriptive study was performed on 90 *E. coli* strains isolated from the children aged from 1 month to 14 years, with urinary tract infection. The resistance patterns of the isolates to different antibiotics were determined by disk diffusion method.

Results:
In this study, the prevalence of the isolates demonstrating a multi-drug resistant phenotype was high. Seventy seven percent of the isolates were resistant to three or more antibiotics. The predominant pattern among these strains (14.4%) included resistance to ampicillin, co-trimoxazole and tetracycline which repeated among 13 strains.

Conclusion:
With due attention to the results and high rate of multi-drug resistance, regular monitoring of antimicrobial drug resistance in different areas is necessary to prevent the unsuitable consumption of the drugs leading to multi-drug resistance.

Keywords: Escherichia coli, Multi-Drug Resistance, Urinary tract infection

Introduction:

Non-intestinal pathogenic *Escherichia coli* (*E. coli*) also known as uropathogenic *E. coli* (UPEC) is the most common cause of urinary tract infection (UTI) in children so that 80% to 90% of the community acquired UTI is caused by this organism (1-3). Prevalence of UTI is not known precisely and it varies according to age and sex. About 8% of girls and 2% of boys at least develop one episode of UTI including pyelonephritis and cystitis during their childhood (4, 5). In the past year, a sharp increase in resistance against antibiotics in *E. coli* isolates have been shown around the world (6). Mutations and transfer of resistance genes by means of transportation are the two main mechanisms responsible for the emergence and spread of bacterial strains resistant to antibiotics. Since a plasmid or transposon can carry several resistance indicators, resistance to several antimicrobial agents might be simultaneously obtained leading to emergence of multi-drug resistant organisms (7-10).

Multi-drug resistant bacteria are those resistant against at least three or more

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symptoms suggestive of lower or upper UTI. After urine sampling from the patients, the samples were immediately transferred to the laboratory for culturing and then were kept at a temperature of 37° C for 24 hours. The culture mediums were assessed after 24 hours and if the organisms had colonies of more than $t10^5$ indicative of UTI, primary diagnostic tests were performed on the colonies. Among the cultured samples those consistent with *E. coli* in terms of culture, biochemistry and morphology were identified as *E. coli*. The sensitivity of each sample to different antibiotics was measured using disk diffusion method and according to the CLSI criteria in 2006 (13). The sensitivity of *E. coli* isolates against 14 antibiotics including gentamicin (10 µg), tetracycline (30 µg), ceftazidime (10 µg), cotrimoxazole (25 µg), imipenem (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cefixime (5 µg), amikacin (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), cefuroxime ((30 µg) and ampicillin (10 µg) were evaluated. *E. coli* ATCC25922 was used as the control.

Results:

Among the collected samples, a total of 90 samples which were consistent with *E. coli* in terms of culture, biochemistry and morphology and identified as *E. coli* were collected and their data were recorded. About 62.5% of the patients were male and the rest were female. The patients' mean age was 21.8±26.9 years. Cystitis was present in 49.2% of the patients while the other had pyelonephritis.

To determine the different patterns of antibiogram, the results of antibiogram testing of the 90 *E. coli* isolates were coded as resistant and sensitive to 14 considered antibiotics. General resistance of the isolates to each of the antibiotics is shown in table 1.

As shown in table 1, the highest resistance was observed in 77 (85.5%) samples antibiotics which are not structurally dependent. Already, increased prevalence of multi-drug resistant and the many resulted problems is one of the concerns of medical society because the treatment is often accompanied with failure and risk of death threatens the patients (11).

Evaluating resistance patterns in E. coli strains which is definitely specific in each region is so important because most of UTIs are resulted by *E. coli* and antibiotics are widely and increasingly used to treat UTI leading to increased resistance in bacteria in addition to emergence of multi-drug resistant bacteria (12). Considering the necessity to identify new or unusual patterns of resistance in *E. coli* and to identify multi-drug resistant organisms, this study was conducted to determine resistance patterns of uropathogenic *E. coli* in Jahrom in order to determine logic therapeutic methods for UTIs in this region and to prevent the increasing prevalence of multi-drug resistant organisms.

Material and Methods:

To conduct this study, *E. coli* strains isolated from urine samples of one month to 14-year-old children with UTI in Motahhari Hospital in Jahrom were collected between 2008 and 2009. UTI diagnosis was confirmed by the medical staff of the hospital on clinical symptoms and laboratory results. Flank pain, fever more than 5/38° C and signs of septicemia in infants was considered as pyelonephritis while dysuria, frequency, bladder pain with mild fever or no fever was mentioned as cystitis. Patients who had community acquired UTI were enrolled in this study; so the children who experienced UTI 48 hours after hospitalization, those with a history of hospitalization one month before sampling and patients who had used antibiotics in the 15 days prior to the sampling were excluded. The patients' data were collected by completing a questionnaire containing questions about sex age, previous history of UTI and

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and imipenem (no sample=0%). Other resistances are exhibited in table 1.

against ampicillin; however, the lowest resistance was against nitrofurantoin (3 samples=3.3%), amikacin (3 samples=3.3%)

Table 1: Frequency of antibiotic resistances of *E. coli* strains isolated from the children with UTI in Jahrom

antibiotic	Resistance frequency among the samples number (percent)	Resistance frequency among multi-drug resistant organisms number (percent)
ampicillin	77 (85.5)	63 (95)
cotrimoxazole	73 (81.1)	62 (93)
tetracycline	68 (75.5)	64 (97)
chloramphenicol	34 (37.7)	33 (50)
nalidixic acid	24 (26.6)	21 (31.8)
cefixime	19 (21.1)	16 (24.2)
cefuroxime	18 (20)	16 (24.2)
gentamicin	15 (16.6)	12 (18.1)
ceftazidime	10 (11.1)	9 (13.6)
ciprofloxacin	8 (8.8)	8 (8.3)
norfloxacin	8 (8.8)	8 (8.3)
nitrofurantoin	3 (3.3)	3 (3.1)
amikacin	3 (3.3)	3 (3.1)
imipenem	0 (0)	0 (0)

antimicrobial drugs and spread of resistant bacteria is obvious (14-16). In previous years increased antibiotic resistance of *E. coli* isolates has been shown around the world which is due to overuse of antimicrobial drugs. In addition to difficulties in treatment, the increased resistance resulted in concerns in different societies including developed and developing countries. For example, cotrimoxazole has been used for treating cystitis in many regions; however, resistance against this drug resulted in using new drugs such as fluoroquinolones and cephalosporines. Unfortunately, resistance against these two antimicrobial agents has been reported by authors, as well (6, 17). Furthermore, resistance against betalactam antibiotics, cotrimoxazole and ampicillin among *E. coli* strains responsible for UTI has been reported in different countries. Gradual increase in resistance against fluoroquinolones is another serious concern which is observed in these strains (18-20).

Since most of patients with UTI especially in the developing countries do not have the economic ability for consulting with a physician or doing laboratory tests and

Forty five resistance patterns were detected among the strains. Thirty seven samples (77%) of the isolates were resistant against three or more antibiotics identified as multi-drug resistant strains. Only 8.3% of the strains were completely sensitive to all of the antibiotics.

Among the 66 samples with multidrug resistance, 19 strains were resistant against three antibiotics (21.1%), 18 strains against four antibiotics (20%), 10 strains against five antibiotics (11.1%), 7 strains against six antibiotics (7.7%) and 12 strains against seven and more antibiotics (13.3%). The most frequent phenotype pattern observed among the samples (14.4%) was resistance against ampicillin, tetracycline and cotrimoxazole with a frequency of among 13 samples.

Besides, the most frequent resistance of the multi-drug resistant organisms was respectively against tetracycline (97%), ampicillin (95%) and cotrimoxazole (93%) (table 1).

Discussion:

The present study showed that 77% of the *E. coli* strains responsible for UTI are resistant against three or more antibiotics. The relation between overuse of

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administration of appropriate antibiotics by physician and a proper supervision, control and time management for using the administered drugs (14).

In the present study, the high frequency of multi-drug resistance among the isolates was considerable; however, about 77% of the samples were resistant against three or more antibiotics. The most frequent drug resistance was observed against ampicillin, tetracycline and cotrimoxazole which were present in 13 samples.

The frequency of multi-drug resistance is different in various countries which is reported to be 7.1% in the United States of America in 2000 (28, 29) and 42% in Slovenia in 2006 (25). The reported frequencies in Iran are different as well, for example 10.9% in Kashan in 2003 and even 65.6% in the recent years (14, 30, 31). This difference between different countries can indicate the difference in the rate of using these drugs in different regions or in the rate of control and supervision on administering antibiotics by therapeutic centers. Moreover, this frequency increases by time passing and inappropriately using drugs.

In the present study, the lowest resistance was against imipenem, amikacin and nitrofurantoin; however, lack of resistance against imipenem has been mentioned by Edvan (32). The high sensitivity to imipenem was also reported by Tarico and Matai which seems to be the drug of choice for treating UTI (26, 33).

In table 2, the frequency of resistance against some of the implemented antibiotics in the treatment of infections due to *E. coli* in different countries is compared. Compared with similar studies in Iran in previous years, in the present study resistance against most of the used antibiotics was significant (14). In addition, regarding the resistance, Iran has the highest frequency of resistance against cotrimoxazole, tetracycline, ceftazidime and chloramphenicol compared with the mentioned countries in table 2 (14, 26, 31). One of the reasons for the increase in multi-drug resistance can be resulted from inevitably are empirically treated, the used drug might not be appropriate for their disease. Different studies are indicative of frequent inappropriate arbitrary administration of many antibiotics even in the developed countries such as the United States of America which is one of the reasons of increased antibiotic resistance in organisms (17, 21, 22).

In the present study the most frequent drug resistance was observed against ampicillin cotrimoxazole and tetracycline which was inconsistent with the World Health Organization guideline about introduction of cotrimoxazole and ampicillin as the drugs of choice in the treatment of UTI in 2007 (23). However, this finding is consistent with the results of many studies in previous years in different countries including developed and developing countries such as Turkey, Senegal, Brazil, Slovenia, South India and Australia (12, 17, 24-27).

The increased resistance might be resulted from inappropriate use of antibiotics in the treatment of infections due to these bacteria and transfer of resistance genes by transportation means such as plasmids of resistance against antibiotics, bacteriophages, transposons and integrons. Since a plasmid or transposon can carry several resistance indicators, resistance against several antimicrobial agents that is simultaneously acquired can result in emergence of multi-drug resistant organisms. For instance, resistance against cotrimoxazole is usually associated with resistance against ampicillin, cephalothin and tetracycline (7-10).

There is agreement about spread of antimicrobial agents and emergence of multi-drug resistant organisms. According to the risk of death among patients infected with multi-drug resistant organisms in addition to higher cost of antibiotics needed for treating them, this group of patients needs more care and hospitalization. Multi-drug resistant organisms have caused many problems in the developing countries which can be reduced and controlled by sufficient timely

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of the strains against this drug is because the patients were infected with *E. coli* strains that were previously resistant and the resistance was not induced electively.

inappropriate use of antibiotics in the treatment of infections due to these bacteria. Regarding the limitation of using tetracycline in children, the high resistance

Table 2: comparison between the resistance against the used antibiotics in the treatment of infections due to *E. coli* in different countries (14, 26, 31)

antibiotic	Percentage of resistance in Iran in the previous years (2003-2007)	Percentage of resistance in Iran in the present study	Percentage of resistance in different countries
cotrimoxazole	48.1	81.1	India (48.3), USA (40.4), Australia (36)
tetracycline	52.4	75.5	India (39.7), Australia (30)
chloramphenicol	-	37.7	India (10.3)
nalidixic acid	16.8	26.6	India (55.2), USA (29.3)
gentamicin	14.8	16.6	India (10.3), USA (8.4), Europe (0.7-7)
ceftazidime	14.1	11.1	India (3.4), Europe (1.6), Spain (0.4), Hungary (0.3), Lebanon (0)
Ciprofloxacin	6.9	8.8	India (22.4), Spain (16), England (6), Denmark (0.7), Canada (0.4)
norfloxacin	18.3	8.8	USA (11), Australia (5.8)
amikacin	6.8	3.3	Europe (1-4), Australia (2.5), India (0), USA (0)
imipenem	1.8	0	India (0), Australia (0), USA (0)

Conclusion: In summary, the findings of the present study show that now a large percentage of *E. coli* strains can be considered as a serious danger in transportation of resistance to general community of *E. coli* and its close bacteria. According to the importance of the subject, it is suggested to conduct larger studies in this issue and to evaluate features of resistant bacteria, their epidemiology and spread by transportation means and its relation with the way of using all common antibiotics. Special attention to this issue is important because many of the current beneficial antibiotics may lose their effectiveness in the treatment of bacterial infections due to lack of attention to resistance mechanisms and especially prevalence and spread of these mechanisms leading to major unsolvable problems in future.

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Unfortunately, in Iran and the third world countries, data about bacterial resistance against antimicrobial agents is so limited; as a result, information about the resistance of these organisms against antimicrobial agents is so beneficial for empirical treatment and using the appropriate drug (14). However, it is obvious that resistance against antibiotics such as ampicillin, cotrimoxazole, tetracycline, ceftazidime and gentamicin used in the treatment of patients infected with *E. coli* is increasing compared with European and American countries. Hence, in order to prevent increasing rate of antibiotic resistance, performing urine culture and antibiotic susceptibility testing is recommended before starting treatment. Furthermore, appropriate antibiotic should be selected according to site of infection, spectrum and pharmacodynamics of drug. To avoid creating resistant strains, unnecessary administration of drugs should be avoided as far as possible and to prevent the spread of infection with resistant strains patient should be isolated before starting treatment.

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