



## Prevalence of Ethiological Factors and Bacterial Sensitivity pattern of Urinary Infection in First and Secondary Graduate Students in Khorramabad City, Iran

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### Abstract

Urinary tract infection (UTI) is one of the most common bacterial infections in childhood that delayed diagnosis and treatment can cause irreversible complications. The aim of this study was to determine the prevalence of urinary tract infection in elementary school children in Khorramabad city, Iran. In this descriptive cross-sectional study, 460 female students of elementary and secondary schools of Khorramabad city were recruited after completing a questionnaire including symptoms of infection, medical and disease history, and urine culture. Antibiotic susceptibility pattern was determined by disk diffusion method accordance with Clinical and Laboratory Standards Institute (CLSI) instructions. Statistical analysis were performed by SPSS software. Of 460 students, based on urine culture results 12 (2.6%) had urinary tract infection. Asymptomatic bacteriuria in 5 patients (1.1%) and positive culture and clinical symptoms were reported in 7 cases (1.5%). *Escherichia coli* (83.3%) was reported as the main cause of UTI among the tested samples. The highest antibiotic susceptibility was related to imipenem (91.6%) and ceftazidime (83.3%). The prevalence of UTI, pathogenic microbial agents and sensitivity to antibiotics in different regions and periods has a significant difference, which can be due to different health levels, constant change in incidence of germs that cause UTI and various antibiotics resistance. Because almost half of the cases of urinary tract infection are asymptomatic, screening for primary school children is necessary.

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### Introduction

Urinary Tract Infection (UTI) is one of the most common bacterial infections in childhood [1, 2] and is the second infection after upper respiratory tract infection which in some cases is associated with fever [3]. UTI can cause child suffering, parent and mother worry, and permanent kidney failure [4]. The potential of the bacteria to cause infection and the severity of the disease depend on the host factors and the virulence of bacteria [3]. Mostly the origin of these infections is the gastrointestinal flora bacteria, the most common Enteropathogenic *Escherichia coli* (E.coli) [6, 5]. Other etiologic factors, such as *Klebsiella* spp, *Proteus*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Pseudomonas*

*aeruginosa* are of secondary importance [7]. Symptomatic urinary tract infection is bacteriuria with clinical symptoms (recurrence and urinary incontinence); in most cases it is accompanied by the presence of white blood cells in the urine (pyuria) [8]. In children younger than 12 years of age, clinical symptoms may be completely nonspecific. Hockenberry et al. (2003) in their basic classification have distinguished symptoms in children aged 0-1 months, 0-24 months, 2-14 years. Anorexia, nausea and growth retardation are common in all three groups of anorexia, and for the 2-14 year-old group, they have reported other possible symptoms, such as abdominal pain and flanks, hematuria, and hypertension. Most of these symptoms point to danger-

ous levels of complications of urinary tract infection [9]. It is estimated that in the first 7 years of life 8% of girls and 2% of boys will experience at least one episode of urinary tract infection, of which 12 to 30% will develop recurrent UTI within a year [10]. Bacterial overgrowth in culture, with or without pyuria, in a person without clinical symptoms is known as "asymptomatic bacteriuria" (ASB) [11]. Asymptomatic bacteriuria occurs in 6% of healthy people. Asymptomatic urinary tract infection is the most common bacterial infection found by doctors in offices and hospitals [12]. Epidemiologically, the prevalence of UTI is strongly correlated with age and gender, so the results show that the prevalence of UTI in the first ten years of birth is 3% in boys and 1% in girls [4]. This infection in males less than one year old was reported to be 2.7% and in females only 0.7%, but in children 2 to 5 years bacteriuria in females increased to 4.5% while in males this rate was higher. It is only 0.5% and this prevalence remains at the age of 15 [13]. In general, girls are about three times more likely to have urinary tract infections than boys [4]. One of the most important causes that can not explain the prevalence of urinary tract infection in girls is its potential to induce vaginal tubes and the ability to accept bacteria through vaginal epithelial cells and thus to cause infections [13,14]. Renal scar as one of the long-term complications of kidney infection can be associated with hypertension and chronic renal failure [15]. Due to the chronic and asymptomatic side effects of this type of infection, it can be considered a risky disease that can lead to long-term complications such as hydronephrosis, cystitis, proteinuria and stones if delayed inappropriate diagnosis and treatment. Urinary tract infection [8]. Unfortunately, the absence of clinical symptoms in patients with asymptomatic bacteriuria can lead to unforeseen complications and consequences, such as progression of the disease to symptomatic cystitis or advanced pyelonephritis [16].

Therefore, timely screening and treatment of UTI is necessary to prevent long-term complications and morbidity thereof [17]. The diagnosis of UTI is based on clinical symptoms, results of urinalysis and urine culture tests (definitive method) [18]. Due to the importance of UTI, especially asymptomatic urinary tract infection in children, numerous studies in Iran and some parts of the world have investigated the frequency of this infection in different epidemiological and bacteriological aspects in primary school girls. On the other hand, the results of various studies have shown that the pattern of microbial susceptibility and etiologic factors of UTI can vary with time and place. In addition, part of the treatment for UTI patients, especially in disadvantaged areas, is empirically done. Therefore, determining the pattern of regional microbial susceptibility can reduce therapeutic failure and prevent complications of infection. Since there have been no documented studies in Lorestan province, we aimed to determine the frequency of UTI (symptomatic and asymptomatic), etiologic factors and microbial susceptibility pattern of isolated bacteria

among in the elementary school girl of Khorramabad city, Iran.

## Materials and methods

### Study Area and Sample Collection

In this cross-sectional descriptive study, the prevalence of UTI was investigated from April to June 2014 in 460 primary and secondary primary school girls in Khorramabad city, Iran.

Sample size using statistical formula:

$$n = \frac{z^2 \times p(1-p)}{d^2}$$

And according to similar studies, taking into account 10% of the sample loss, 460 samples were determined. For the purpose of sampling, 10 elementary schools (public and non-profit) were selected from different educational districts of north, south, east, west and downtown and each elementary school enrolled 46 primary and secondary students by multistage random sampling method. Students with a specific underlying disease, antibiotic intake three days before sampling, and parental dissatisfaction were excluded. Variables such as demographic information, clinical symptoms, history of some diseases, etc. were asked by parents of students and were recorded in a questionnaire. Then, parents were taught how to collect urine samples correctly. The training included rinsing the perineum with soap and water and collecting the middle of the students' morning urine. Urine samples were collected in sterile containers and sent to the laboratory on ice.

### Urine culture and analysis

First, the urine samples were collected, cultured on MacConkey Agar and Blood Agar medium (Merck, Germany) and then tested for urine analysis. However, the number of colonies grown on blood agar medium was more than 105 CFU / ml as typical urinary tract infection. Colony counts less than 105 CFU / ml were interpreted according to standard guidelines and microbiological references. Also, in urine analysis experiments, on average, the presence of at least 5 leukocytes per microscopic field (x40) was considered as pyuria.

### Determination of antibiotic susceptibility pattern

Antibiotic susceptibility of the strains was determined by disk diffusion method (Kirby-Boer) on Müller Hinton agar medium (Merck, Germany) and in accordance with CLSI (Clinical and Laboratory Standards Institute) guidelines. Antibiotic discs used (Mast, UK) in this study including cefazolin (30µg), gentamicin (10µg), amikacin (30µg), nitrofurantoin (300µg), nalidixic acid, imipenem (10µg), ceftazidime (30µg), coadministration 1.25/23.75 µg), ciprofloxacin (5 µg), was cefotaxime.

### Statistical analysis

Descriptive statistics, ratios and percentages were calculated using SPSS software and chi-square test for data analysis. In all cases,  $p < 0.05$  was considered significant.

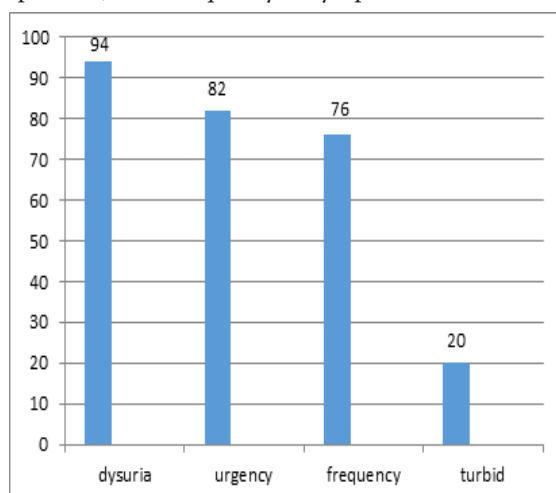
### Ethical Considerations

Written informed consent was obtained from all parents of the students studied according to the Ethics Committee of the

Vice Chancellor for Research and Technology of Lorestan University of Medical Sciences. Parents were also informed about the results of the tests.

## Results

In this descriptive cross-sectional study, 460 primary and secondary primary female students were studied for prevalence of UTI. From 460 samples, 189 (42%) were first grade and 261 (58%) were second grade. According to the results of the questionnaire completed by parents, 260 (56.5%) had no symptoms of UTI while 200 (43.5%) of students had at least one infection-related symptoms. The most commonly reported symptoms were urinary irritation 94 (20.4%) followed by urinary urgency 82 (17.8%) and the least common symptom was urinary color opacity (20 patients). The frequency of symptoms is shown in Fig 1.



**Figure 1.** Frequency (number) of clinical symptoms in the students studied (Number: 460)

According to the results of urine culture, 12 (2.7%) of the 460 students studied had urinary tract infection and at least one gram-negative bacillus with  $\leq 10^5$  CFU / ml colonies was isolated from their urine culture. Of the 189 first-grade students, 6 (2.3%) and of the 261 second-grade students, 6 (2.3%) had positive urine cultures and had UTI. There was no statistically significant difference in the prevalence of UTI according to the educational level of students ( $p = 0.556$ ). The prevalence of UTI by age, parental education, risk factors for UTI (history of hospitalization, previous history of UTI) is shown in Table 1.

**Table 1.** Comparison of prevalence of UTI among students, by demographic characteristics and clinical history

| Urinary Tract Infection/<br>Variable | Comparison of affected<br>and non-affected cases | Total      | Not at all<br>448 = n | Affected<br>12 = n |
|--------------------------------------|--|------------|-----------------------|--------------------|
|                                      | p- value   | Number (%) | Number (%)            | Number (%)         |
| Age                                  |  |            |                       |                    |
| 5-7 years                            | 0.405  | 196(100)   | 190(96.9)             | 6(3.1)             |
| 8-9 years                            |  | 264(100)   | 258(97.7)             | 6(2.3)             |
| Mother's education                   |  |            |                       |                    |
| illiterate                           | 0.232  | 29(100)    | 29(100)               | 0(0)               |
| Non-academic                         |  | 320(100)   | 309(96.6)             | 11(3.4)            |
| Academic                             |  | 111(100)   | 110(99.1)             | 1(0.9)             |
| Father's education                   |  |            |                       |                    |
| illiterate                           | 0.143  | 30(100)    | 28(93.3)              | 2(6.7)             |
| Non-academic                         |  | 299(100)   | 290(97)               | 9(3)               |
| Academic                             |  | 131(100)   | 130(99.1)             | 1(0.8)             |
| History of hospitalization           |  |            |                       |                    |
| Has it                               | 0.743  | 62(100)    | 60(96.8)              | 2(3.2)             |
| Does not have                        |  | 398(100)   | 388(97.5)             | 10(2.5)            |
| Previous history of UTI              |  |            |                       |                    |
| Has it                               | 0.005  | 14(100)    | 12(85.7)              | 2(14.3)            |
| Does not have                        |  | 446(100)   | 436(97.8)             | 10(2.2)            |

Among patients with urinary tract infection ( $n = 12$ ), 4 (33.3%) had the most common symptom. The incidence of urinary irritation in children without UTI was 90 (20.1%). The difference in incidence of urinary incontinence between the two groups of children with and without urinary tract infection was not statistically significant ( $p = 0.226$ ).

All the students under study were classified in different groups according to the results of microbial culture, pyuria and clinical symptoms according to microbiological references. Most of the subjects (48.9%) had no bacteriuria, pyuria or

clinical symptoms. 26 (5.6%) had sterile pyuria. Asymptomatic bacteriuria was reported in 5 (1.1%) patients. Seven (1.6%) patients showed positive culture and clinical signs suggesting the possibility of cystitis. In other students (42.8%), despite clinical symptoms, urine culture was negative, suggesting the possibility of infection with other pathogens such as Chlamydia, cytomegalovirus, adenovirus, and gonococcus.

A comparison of the frequency distribution of other clinical and laboratory symptoms between the affected and non-infected groups is summarized in Table 2.

**Table 1.** Comparison of prevalence of UTI among students, by demographic characteristics and clinical history

| Urinary Tract Infection/<br>Variable | Comparison of affected<br>and non-affected cases | Not at all<br>448 = n | Affected<br>12 = n |
|--------------------------------------|--|-----------------------|--------------------|
|                                      | p- value   | Number (%)            | Number (%)         |
| Urinary irritation                   |  |                       |                    |
| Has it                               | 0.261  | 90(20.1)              | 4(33.3)            |
| Does not have                        |  | 358(79.9)             | 8(66.7)            |
| Frequent urination                   |  |                       |                    |
| Has it                               | 0.118  | 76(17)                | 0(0)               |
| Does not have                        |  | 372(83)               | 12(100)            |
| Urgency in urination                 |  |                       |                    |
| Has it                               | 0.915  | 80(17.9)              | 2(16.7)            |
| Does not have                        |  | 368(82.1)             | 10(83.3)           |
| Urine color opacity                  |  |                       |                    |
| Has it                               | 0.417  | 19(4.2)               | 1(8.3)             |
| Does not have                        |  | 429(95.8)             | 11(91.7)           |

## Etiological Factors of Urinary Tract Infection (UTI)

In patients with UTI, E.coli was reported as pathogen in 10 cases (83.3%), Enterobacter and Klebsiella in 1 case (8.3%) (Table 3).

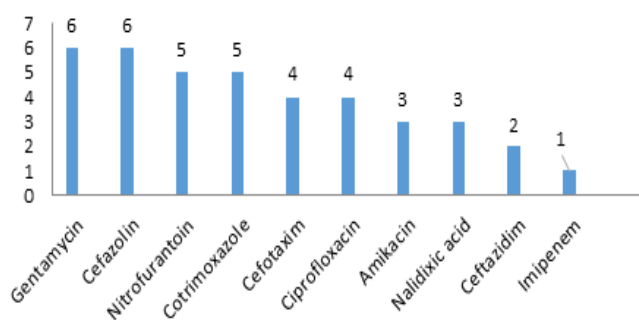
## Antimicrobial Susceptibility Pattern

The results of microbial susceptibility analysis showed that the highest antibiotic susceptibility to imipenem (91.6%) and ceftazidime (83.3%) and the highest resistance to cefazolin (50%) and gentamicin (50%) respectively (Fig 2).

**Table 3.** Antibiotic resistance among 12 bacterial isolates isolated from culture of UTIs

| Infection factor      | Number | Cefazolin | Cefotaxime | Ceftazidime | Ciprofloxacin | Cotrimoxazole | Gentamicin | Nalidixic Acid | Nitrofurantoin | Imipenem | Amikacin |
|-----------------------|--------|-----------|------------|-------------|---------------|---------------|------------|----------------|----------------|----------|----------|
| E. coli               | 10     | 5(5)      | 3(30)      | 2(20)       | 4(40)         | 5(50)         | 5(50)      | 3(30)          | 3(30)          | 1(10)    | 3(20)    |
| Klebsiella pneumoniae | 1      | 0(0)      | 0(0)       | 0(0)        | 0(0)          | 0(0)          | 0(0)       | 0(0)           | 1(100)         | 0(0)     | 0(0)     |
| Enterobacter          | 1      | 1(100)    | 1(100)     | 0(0)        | 0(0)          | 0(0)          | 1(100)     | 0(0)           | 1(100)         | 0(0)     | 0(0)     |
| Total (%)             | 12     | 6         | 4          | 2           | 4             | 5             | 6          | 3              | 5              | 1        | 3        |





**Figure 2.** Antibiotic resistance pattern of 12 bacterial isolates (*E. coli*, *Enterobacter* and *Klebsiella*) obtained from the urine culture of primary and secondary primary female students studied (N)

## Discussion

In this study, the prevalence of urinary tract infection was 2.6%, compared to Mohammed's study in Egypt with the aim of examining the prevalence of bacteriuria in children without Signs differed in the number of 1000 students aged 6-12 years (552 boys, 448 girls) in a cross-sectional study. In that study, the prevalence of bacteriuria was estimated to be 6%, which was 11.4% in girls compared to 1.6% in boys [19], which was much higher than girls in our study. However, the results of our study were more similar to studies in Iran. In the study of Rostami et al. On primary school children in Ardabil this rate (4.2%) was higher than the present study [20]. In the study of Elahi et al., Which was conducted on female students of elementary school in Turkmenistan, reported prevalence of 2.3% [21], which is consistent with our study. This rate was 1.1% in our study, which is lower than our study, which may be due to the low sample size, although the prevalence of bacteriuria in girls was not reported separately, and given that half of the subjects were Studies have been boys, and urinary tract infections are less common among boys [22], which may be the cause of this discrepancy. However, in the Haji Amini study on female samples, the prevalence of UTI was reported to be 15.1% [23], while Kamali et al reported a 6.8% prevalence of UTI in screening female students in Zanjan. [24] which is significantly different from the results of the present study; it appears that part of this difference was due to a colony count of more than 104 CFU/ml for urinary tract infection; but in the present study, positive samples, colony counts were more than 105 CFU/ml [23].

In this study, 26 (5.6%) had sterile pyuria. Asymptomatic bacteriuria was reported in 5 (1.1%) patients. Seven (1.6%) patients showed positive culture and clinical signs suggesting the possibility of cystitis. In 194 cases (43.3%), despite clinical signs, urine culture was negative, in which cases infection with other etiologic factors such as Chlamydia, Mycoplasma, cytomegalovirus, adenovirus, etc., as well as infectious causes such as structural problems of the genital tract. It may be argued that definitive diagnosis requires more careful laboratory and other follow-up investigations.

Asymptomatic bacteriuria accounted for 1.1% in the present study, while this was higher in other studies. In a study in Greece, the prevalence of asymptomatic bacteriuria among children was 7% [24]. In two other studies on children 4 to 16 years and 7 years of age this rate was 8% and 5.4%, respectively (25, 24). Among our subjects, 34 (7.3%) had pyuria, of which 8 (23.5%) had positive culture and 26 (76.5%) had negative pyuria. The prevalence

of pyuria was 11 (1.7%) in all of the subjects studied, of whom 6 had UTI and 5 (45.5%) had sterile pyuria [21]. In our study, 7 patients (58.3%) had urinary tract infection and the rest did not report any symptoms. In a study conducted by Fesharaki et al, on students in one of Tehran's cities, 64.28% had no symptoms and the rest had at least one symptom of UTI such as burning, frequent urination, abdominal pain and etc., have been consistent with the recent study [22]. In 194 (43.3%) of those who had negative urine cultures, clinical symptoms were noted, including infection with other etiologic factors of UTI such as chlamydia, mycoplasma, cytomegalovirus, adenovirus, etc., as well as infectious causes such as structural problems. The genital tract can be raised; definitive diagnosis requires closer laboratory testing and other follow-up.

In this study, the most common etiologic factor for UTI was *E. coli* (83.3%), which is consistent with the results of many similar studies [20-23]. In the Mohammed study, *E. coli* was found in 35 cases (58% of the sample), including 3 boys and 32 girls. In the population of girls with UTIs, 62.7% of cases were *E. coli*. In Mohammed's study, *Staphylococcus aureus* was found in 13 cases (22%) including 3 boys and 10 girls, *Enterobacter* in 10% of samples including 6 girls, *Klebsiella pneumoniae* in 5% of cases including 3 boys and *Proteus vulgaris* in 5% of samples included 3 girls [19]. In the study of Fesharaki and Farajolah, all of the microorganisms grown were *E. coli* [21, 22]. In our study, *Enterobacter* and *Klebsiella* were the second most common pathogens with 8.3% for both bacteria. In the study of Haji Amini, *Proteus* and *Klebsiella* had the highest prevalence after *E. coli*, each with 7% (23). In Mohammed's study, the next rank was for *Staphylococcus aureus* after *E. coli* [19].

Among the isolated bacterial agents, the highest antibiotic susceptibility to imipenem (91.6%) and ceftazidime (83.3%) and the highest resistance to cefazolin (50%) and gentamicin (50%) respectively which contradicts most studies. For example, the highest sensitivity to nalidixic acid (84.97% and 92.30%, respectively) was reported by Saraj et al, in Ahwaz and Madani et al, in Kermanshah, respectively [25, 26]. Nitrofurantoin (92.3%) and amikacin (66.67%) had the highest sensitivity in the Soureshjani study [27].

In Fesharaki study, ceftriaxone (71.4%) was most sensitive [22]. In our study, cefazolin with 50% resistance and gentamicin with 50% resistance were the most resistant to antibiotics. This result contradicts the work of others. In the Soureshjani and Madani studies, ampicillin was identified as the most unsuitable antibiotic with 85.71% resistance [26, 27].

In the study of Ferdosi-Shahandashti et al., The effect of different antibiotics on *E. coli* was investigated and it was reported that *E. coli* is most susceptible to imipenem, afloxacin and ciprofloxacin [28] which is similar to imipenem in our study.

According to numerous reports, statistics in different regions and times show that the microbial pathogens and their susceptibility to antibiotics are significantly different. These differences may be due to the constant change in the incidence of UTIs and the susceptibility and resistance to different antibiotics.

The results of the study of factors affecting UTI showed that in both affected and untreated groups, father ( $p = 0.094$ ) and mother ( $p = 0.248$ ) were uneducated but compared to the two groups. The amount was not significant. Therefore, low levels of parental literacy in relation to UTI cannot be generalized. Among other risk factors for UTI, history of hospitalization was statistically significant between the two groups ( $p = 0.005$ ).

## Conclusion

Considering asymptomatic UTIs in approximately half of the cases of UTIs in primary school girls in this study and its complications and consequences, which can sometimes be irreversible, minimum annual screening as well as training of factors affecting UTI and its prevention. Students and their parents need to improve community health.

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## Authors' contribution

All authors contributed equally to the manuscript.

## Conflicts of interest

The authors declared no competing interests.

## Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication and etc.) have been completely observed by author.

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