Choosing the correct empirical antibiotic for urinary tract infection in pediatric: Surveillance of antimicrobial susceptibility pattern of *Escherichia coli* by E-Test method

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ABSTRACT

Background and Objectives: Urinary Tract Infections (UTIs) are among the most common bacterial diseases worldwide. We investigate the antibiotic susceptibility patterns of *Escherichia coli* (*E. coli*) strains isolated from pediatric patients with community acquired urinary tract infection (UTI) to find a clinical guidance for choosing a right empirical antibiotic in these patients.

Materials and Methods: In this cross sectional study, 100 urine specimens which were positive for *E. coli* had been investigated for antibiotics susceptibility pattern. The susceptibility to Co-trimoxazol (25µg), Amikacin (30µg), Ceftriaxone (30µg), Nalidixic Acid (30µg), Cefixime (5µg), and Nitrofurantoin (300µg) tested with Disk diffusion agar and MIC determined with the E-test.

Results: Mean age of patients was 38 Months. Girls had greater proportion than boys (74 versus 26%). In Disk diffusion method, 26% of the isolates were susceptible to cotrimoxazole. Susceptibility to amikacin, ceftriaxone, nitrofurantoin, nalidixic acid and cefixime was 94%, 66%, 97%, 62% and 52%, respectively. By E-Test method and according to CLSI criteria susceptibility for co-trimoxazol, amikacin, ceftriaxone and nalidixic acid was 37%, 97%, 67% and 50%, respectively. The highest percentage of agreement between Disk diffusion and E-Test method was found for amikacin (96%) and the lowest percentage for co-trimoxazole (89%).

Conclusions: Treatment failure, prolonged or repeated hospitalization, increased costs of care, and increased mortality are some consequence of bacterial resistance in UTIs. Misuse of antibiotics in each geographic location directly affects antibiotic resistance pattern. In the treatment of UTI, proper selection of antimicrobial agents should be relevant to the bacterial susceptibility testing surveillance. According to our results, amikacin as an injectable drug and nitrofurantoin as an oral agent could be used as a drug of choice in our region for children with UTIs.

Keywords: Antibiotic susceptibility, *Escherichia coli*, Urinary Tract Infections, Disk diffusion agar, E-Test
agent should be adjusted by local susceptibility pattern. The rising prevalence of multi-drug resistant infections makes more complexity in the empirical treatment of these infections (2). The antimicrobial resistance patterns of community-acquired UTIs have been studied by some researcher around the world (1, 2). Awareness of local antimicrobial agent’s resistance trends among urinary isolates is important to notice clinicians for appropriate use of antibiotics and also for evidence based recommendations in empirical antibiotic treatment of UTI. (3-6)

Proper application of sensitive and specific methods in determination of susceptibility of antimicrobial agents is necessary for exact diagnosis and early effective treatment. Determination of antibiotic susceptibility patterns of E. coli strains is one of the most concerns in empirical management of community acquired UTIs in pediatric practice.

This study was conducted to determine the minimal inhibitory concentration (MIC) of urinary E. coli isolates by E-test.

MATERIALS AND METHODS

Bacterial Strains. This cross-sectional descriptive study was conducted between June 2009 and September 2010. A total of 100 E. coli (one specimen per patient) isolated from children admitted with community-acquired UTIs at the Besat teaching Hospital. Choosing the right method of specimen collecting was according to physician decision, clinical status and need for treatment in patients. Supra-pubic, catheter and midstream sampling have been utilized in this study. After appropriate sampling, specimen were sent to the microbiology laboratory in a standard manner and processed by trained personnel. Isolation performed according to standard laboratory protocols and Uropathogenic Escherichia coli (UPEC) isolated from true positive urine cultures.

Diagnostic criteria. Community acquired urinary tract infections were defined as recovery of 100 000 CFU/mL or more bacteria in midstream clean-catch method, 50 000 CFU/mL or more by catheter and any count by supra-pubic rout (7).

Antimicrobial Susceptibility Testing. Antimicrobial sensitivity testing to the following four antimicrobial agents was performed with both Disk diffusion agar (Mast Co, UK) and E-Test (AB biomerienx solna Sweden), according to the Clinical and Laboratory Standards Institute (CLSI) criteria (8): Co-trimoxazol (25µg), Amikacin (30µg), Ceftriaxone (30µg) and Nalidixic acid (30µg). For Cefixime (5µg) and Nitrofurantoin (300µg) only Disk diffusion agar were utilized. This study was approved by the Research Committee of Hamadan University of Medical Sciences.

Statistical Analysis. All data were processed using Statistical Package for Social Sciences (SPSS), version 17.0 software. The significance of the results was established using MC- Nemar Test. the level of significance was considered at \( P <0.05 \).

RESULTS

During 14 months surveillance period 100 Escherichia coli isolated from urine specimens of patients with clinically and laboratory diagnosis of UTI were investigated for antibiotic susceptibility testing. Mean age of patients was 38 Months. Girls had greater proportion than boys (74 versus 26%). Among patients who have positive culture for E. coli, E-test susceptibility testing was performed and the following results obtained: 37% susceptible

### Table 1. Antimicrobial resistance of E. coli isolates by E. test (n = 100)

| Antibiotic         | Break point(µg/ml) | Resistant | Intermediate | Susceptible | MIC-50 | MIC-90 | MIC Range |
|--------------------|--------------------|-----------|--------------|-------------|--------|--------|-----------|
| Co-trimoxazole     | ≤ 8                | 63%       | 0%           | 37%         | 0.094  | 0.61   | 0.016-2   |
|                    | ≥ 32               |           |              |             |        |        |           |
| Nalidixic Acid     | ≤ 8                | 42%       | 8%           | 50%         | 2      | 3      | 1-192     |
|                    | ≥ 32               |           |              |             |        |        |           |
| Ceftriaxone        | ≤ 8                | 21%       | 12%          | 67%         | 0.032  | 0.2    | 0.016-128 |
|                    | ≥ 64               |           |              |             |        |        |           |
| Amikacin           | ≤ 16               | 3%        | 0%           | 97%         | 0.75   | 1.6    | 0.125-32  |
|                    | ≥ 32               |           |              |             |        |        |           |
to Co-trimoxazole, 50% susceptible to Nalidixic Acid, 67% susceptible to ceftriaxone and 97% to Amikacin (Table 1).

In Disk diffusion agar test, Drug susceptibility of the isolates was 66%, 94%, 62%, and 97% to ceftriaxone, Amikacin, cefixime, and Nitrofurantoin, respectively (Table 2).

Co-trimoxazol obtained the lowest and Amikacin and Nitrofurantoin had the highest sensitivity with both methods which were used in our study.

According to the MICs of co-trimoxazol (MIC≥32), 63% of *E. coli* strains were classified as resistance. The MICs of other antimicrobial agents showed that *E. coli* strains were highly susceptible to Amikacin. Their MIC<sub>90</sub> was 1.6 µg/ml.

The highest and the lowest overall agreement were found between E-Test and Disk diffusion agar test for amikacin (96%) and co-trimoxazol (89%), respectively (Table 3).

In comparison with results of E-Test susceptibility testing, no statistically significant differences were found in Disk diffusion agar test, with the exception of co-trimoxazol (*P*-value<0.05).

### DISCUSSION

Understanding the impacts of drug susceptibility pattern is crucial as the changing rate of antibiotic susceptibility has a large impact on the treatment of UTI. Treatment failure, prolonged or repeated hospitalization, increased costs of care, and increased mortality are some consequence of bacterial resistance in UTIs. Although, several factors (such as bacterial virulence, bacterial biofilms formation in urinary tract, previous inappropriate use of antimicrobial agents and structural anomalies) may play a role in antimicrobial resistance, misuse of antibiotics in each geographic location directly affects antibiotic resistance pattern.

The purpose of this study was to investigate the antimicrobial susceptibility pattern of uropathogenic *E. coli* in pediatric patients in our province for better usage of empiric antimicrobial agents. Sex distribution in our study was compatible to other study and reference textbooks (1, 9). The lowest sensitivity result, or the most resistance, belongs to Co-trimoxazole in both Disk diffusion agar

### Table 2. Antimicrobial resistance of *E. coli* isolates by Disk Diffusion Agar (n = 100)

| Antibiotic       | Susceptible (%) | Intermediate (%) | Resistance (%) |
|------------------|-----------------|------------------|----------------|
| Co-trimoxazole   | 26 (26)         | 4 (4)            | 70 (70)        |
| Cefixime         | 62 (62)         | 3 (3)            | 35 (35)        |
| Nalidixic Acid   | 52 (52)         | 1 (1)            | 47 (47)        |
| Nitrofurantoin   | 97 (97)         | 3 (3)            | 0 (0)          |
| Ceftriaxone      | 66 (66)         | 4 (4)            | 30 (30)        |
| Amikacin         | 94 (94)         | 2 (2)            | 4 (4)          |

### Table 3. Comparison of Antimicrobial susceptibility pattern of *E. coli* isolates by Disk Diffusion Agar and E. test (n = 100)

| Antibiotic       | Disk Diffusion Agar (%) | E-Test (%) | *P*-Value |
|------------------|-------------------------|------------|-----------|
| Co-trimoxazole   | 26 (26%)                | 37 (37%)   | 0.001     |
| Nalidixic Acid   | 52 (52%)                | 50 (50%)   | 0.500     |
| Ceftriaxone      | 66 (66%)                | 67 (67%)   | 1.000     |
| Amikacin         | 94 (94%)                | 97 (97%)   | 0.250     |
and E-Test (susceptibility rates were 26 and 37%; respectively). Same results reported by other study in our country such as Khorvash et al. 2008 (10), Prais et al. 2002 (11) and Farajnia et al. 2009 (9).

Nitrofurantoin, after Amikacin, has attained subsequent rank of susceptibility (97% by Disk diffusion agar test). These results were same to other study in our country (12-14), and is comparable to the 94.9% susceptible E. coli isolated from 240 recurrent UTIs other studies (15-18).

Interestingly, one of the most relevant antibiotics, ceftriaxone had changed susceptibility pattern in the base of previous findings demonstrate that reported sensitivity were more than 90% (12, 16). In our study, sensitivity of ceftriaxone as determined as 66% and 67% in Disk diffusion agar and E-Tests, respectively. The reduced sensitivity may be consequence of unbridle use of this agent in outpatient as well as inpatient management of pediatric infectious disease (19, 20). On the other hand, emergence of community-acquired E. coli producing extended spectrum beta-lactamases (ESBL) should be keep in mind and could be scope of further investigation for researchers (21, 22). These E. coli producing beta-lactamases also could exhibit co-resistance to co-trimoxazole (15). Moyo et al. (23) showed more resistance to co-trimoxazole and nalidixic acid than previous reports (more than 90% and 61%, respectively). Noemia et al. (24) proposed initial empirical oral treatment with nitrofurantoin or nalidixic acid in children with febrile UTI, but according to finding of our study nalidixic acid could not be the drug of choice in our region. Sensitivity of nalidixic acid in other studies was greater than our survey (18).

We conclude that former agents such as co-trimoxazole and nalidixic acid which have been used widely had more resistant rate. On the other hand, those (nitrofurantoin, amikacin) with lesser usage have the little resistant rate. Whereas E. coli is the most common uropathogen in pediatric patients (18, 25), it is advisable that co-trimoxazole and nalidixic acid do not used routinely for empiric practice. In afebrile UTIs (lower UTI or cystitis) and also in vesicourethral reflux prophyaxis, nitrofurantoin could be considered. If patient is febrile (upper UTI) and no concern exists about renal function, amikacin will be the drug of choice because of its high efficacy to E. coli. Another advantage of amikacin in primary treatment of febrile UTI in contrast with ceftriaxone is that ceftriaxone can induce beta-lactamase resistance instead of in vitro sensitivity which may cause treatment failure. Authors recommended that pediatrician should review antibiotic resistance pattern in their region and investigate susceptibility of oral and parenteral agents, as this study revealed that empirical initial treatment with co-trimoxazole is inadequate in approximately one third of UTI cases. Notable in this context is that in comparison between results of E-Test and Disk diffusion agar, no statistically significant differences were found, with the exception of co-trimoxazol (P.value <0.05) which implies that antibiotic resistance for co-trimoxazol over estimated by Disk diffusion method but this method is reliable for other antibiotics (nitrofurantoin, nalidixic acid and amikacin).

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