ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESCHERICHIA COLI ISOLATED FROM CHILDREN WITH URINARY TRACT INFECTION

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INTRODUCTION

The urinary tract infection (UTI) is the colonization of a pathogen occurring anywhere in the urinary tract (kidney, ureter, bladder, and urethra). UTI is a common condition in children. Approximately 1 in 10 girls and 1 in 30 boys will have a UTI by the age of 16 years [1,2]. During UTI, multiplication of the organism takes place in the urinary tract and there is the presence of more than a hundred thousand organisms in a 1-mL urine sample [3]. The most common pathogen is Escherichia coli, accounting for approximately 85% of UTIs in children. Renal parenchymal defects are present in 3–15% of children within 1–2 years of their first diagnosed UTI [4]. Around 90% of UTIs were caused by E. coli [5].

The most common symptoms of UTI are dysuria, frequency, and urgency in urination, suprapubic pain, and possible hematuria. The urine may have an unpleasant odor and appear cloudy. When UTI will persist in children for more than a week with systemic symptoms of persistent fever, chills, nausea, and vomiting [6]. Antibiotics susceptibility test measures the ability of an antibiotic or another antimicrobial agent to inhibit the growth of microorganisms [7]. The choice of use of antibiotics and the duration of treatment depend on the history of the patient and the bacterial agent identified. For uncomplicated UTI disease is cured with 1 or 2 days of treatment and to ensure that the infection is cured antibiotics should be taken within a week or 2 weeks [8]. The objective of this research is to identify the incidence of E. coli causing UTI in children and determine the antibiotic susceptibility pattern of the isolates.

METHODS

Sample collection and transport

The urine samples were collected by suprapubic bladder aspiration and midstream urine sample methods. In the suprapubic bladder aspiration method, urine was withdrawn directly into a syringe through a percutaneously inserted needle, thereby ensuring contamination-free specimen [9]. The midstream urine samples were collected from children who are toilet trained. Children are instructed to clean the periurethral area and were asked to pass urine at first by allowing and losing some urine and a midstream urine sample was collected in a sterile container. The collected urine sample was transported as soon as possible and 1.8% boric acid was added in case of delay.

Sample processing

The urine sample was processed by macroscopic and microscopic examination. The macroscopic examination observed the color and turbidity. In a microscopic examination, 5–10 mL of urine sample was centrifuged at 3000 rpm for 10 min and the sediment was observed under a microscope [10].

Culture of the urine sample

The culture of urine specimens was done on 5% blood agar (BA), MacConkey agar using a semiquantitative culture method [10]. About 0.001 mL of urine sample was streaked in culture media using a sterile inoculating loop and incubated in an inverted position at 37°C for 24 h. The photograph of a pure culture of E. coli in BA was shown in Fig. 1.

Identification of isolates

Identification of major isolates was done using standard microbiological techniques as described in the Bergey’s manual that involves the morphological appearance of colonies, staining reactions, and biochemical properties such as Gram’s staining, catalase, oxidase, coagulase, indole, methyl red, Voges-Proskauer, citrate utilization, triple sugar iron, urease, and sulfide indole motility tests.

RESULTS

Out of a total of 530 samples, 114 (21.50%) showed significant growth. A total of 8 different types of bacteria were isolated from the growth of positive samples. Among the isolates, E. coli 66 (57.8%) was found to be the most predominant organism followed by Klebsiella pneumoniae 18(15.8%), Proteus spp. 10 (8.8%), Staphylococcus aureus 8 (7.0%), Acinetobacter spp. 4 (3.5%), CoNS 4 (3.5%), Enterobacter spp. 2 (1.8%), and Pseudomonas aeruginosa 2 (1.8%). In the present study, out of 66 E. coli, 37 (56.1%) were multidrug-resistant strain. E. coli showed 94.0% resistance to ceftriaxone followed by ceftazi dime 86.5% and cefotaxime 70.3%. Imipenem (91.9%) followed by amikacin (89.2%) seems to be the effective drug against UTI causing E. coli in children.

Conclusion:

Multidrug resistance may possess difficulties with the choice of therapeutic options for the treatment of severe infections.

Keywords: Antimicrobial resistance, Ceftriaxone, Multidrug-resistant, Staphylococcus aureus.

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Antibiotics susceptibility test
The antimicrobial susceptibility test of isolates was done by modified Kirby–Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute [11] Wayne, USA, using Muller–Hinton Agar (MHA). The isolated colonies were transferred to the nutrient broth and were incubated for 4 h until the turbidity of bacterial growth was similar to that of 0.5 McFarland standards. The sterile cotton swab was dipped into the tube containing culture and inoculated over dried MHA by carpet culture technique. About six antimicrobial disks each 6 mm in diameter were placed on the inoculated plates on a 90 mm diameter plate. The plates were left at room temperature for the diffusion of antibiotics from the disk. It was incubated at 37°C for 18 h and the plates were examined to ensure confluent growth and the diameter of each zone of inhibition in mm was measured [8].

Multidrug-resistant (MDR) analysis
The multidrug-resistant strain was evaluated using the Kirby–Bauer disk diffusion technique [10]. Those isolates which showed resistance to 3 or more than 3 groups of antibiotics were considered multidrug-resistant [12]. Different types of drug in *E. coli* are shown in Fig. 2.

Quality control
The standard protocol was followed to maintain the quality of each test. All the agar plates were incubated at 37°C for 24 h before use to examine for any contamination that occurred during media preparation and storage. The antibiotic susceptibility test of the isolates was standardized using control strains preserved in the hospital.

Purity plate and statistical analysis
The purity plate was used to ensure the inoculation for the biochemical tests is pure culture and also to confirm whether the biochemical tests performed are in an aseptic condition or not. Before and after performing biochemical tests, the same inoculum was subcultured in nutrient agar media and incubated. It was observed for the appearance of pure growth of organisms. The measurements were statistically analyzed using Statistical Package for the Social Science version 20 software packages. Scheme 1 represents the flow sheet for the urine samples for the detection of UTI.

RESULTS AND DISCUSSION
Five hundred thirty urine samples were collected from the suspected UTI patients at International Friendship Children Hospital spanning
between September 2015 and April 2016 to detect the incidence and antibiotic susceptibility pattern in E. coli.

Distribution of bacterial isolates in total processed samples
Out of the total 530 samples, 114 (21.50%) showed significant growth. A total of 8 different types of bacteria were isolated from positive samples.

In Gram-negative bacteria, E. coli was the dominant organism 66 (57.8%) followed by Klebsiella pneumoniae 18 (15.8%), Proteus spp. 10 (8.8%), Acinetobacter spp. 4 (3.5%), Pseudomonas aeruginosa 2 (1.8%), and spp. 2 (1.8%). Gram-positive bacteria, Staphylococcus aureus accounts for 8 (7%) and CoNS 4 (3.5%), respectively. The growth pattern of bacteria in the sample is shown in Fig. 3 and Table 1.

Growth of E. coli in urine samples and gender-wise distribution patterns
Fig. 4 shows the distribution pattern of E. coli in urine samples collected from male and female children. Among 114 samples having major uropathogens, 66 (57.8%) bacteria isolates were found to be E. coli. Out of 66 E. coli isolates, the maximum number of E. coli was distributed in females 40 (60.6%) as compared to 26 (39.4%) in males.

Children age and gender-dependent distribution of E. coli in patients
Children age and patient gender-dependent distribution cases of E. coli are as shown in Table 2. The highest susceptible children age group of patients out of 26 cases, E. coli isolated in male was 1–5 years (n=28, 42.5%) followed by age group below 1 year (n=6, 23.1%), 5–10 years (n=4, 15.4%), and 10–15 year 1 (n=1, 3.8%), respectively. While in females, the highest susceptible (n=13, 32.5%) was observed in age group 1–5 years, followed by age group below 1 year (n=9, 22.5%), 5–10 year (n=8, 20%), 10-15 (n=6, 20%), and above 15 year (n=2, 5%), respectively. Statistically the result is insignificant (p>0.05) as in Table 2.

Distribution frequency of E. coli in different hospital departments
The distribution of E. coli in different hospital departments is presented in Table 3. Among the total of 530 urine samples, 375 (70.8%) and 155 (29.2%) samples were from outdoor and indoor patients, respectively. Culture positive cases for E. coli for indoor and outdoor patients were 32 (48.5%) and 34 (51.5%), respectively.

Antibiotic susceptibility pattern of E. coli in first- and second-line drugs in children
Among the seven first-line drugs used in patients, E. coli was found to be resistant against ceftriaxone (94.0%) followed by gentamicin (66.7%), ampicillin (65.2%), and cotrimoxazole (63.6%), respectively. While nitrofurantoin was found more sensitive among first-line drugs with (77.3%) sensitivity followed by norfloxacin (68.2%) and ciprofloxacin (54.5%), respectively, as shown in Table 4.

Among 66 isolated E. coli, 37 (56.1%) were multi drug resistant to first-line drugs. Hence, an antibiotic susceptibility test of second-line drugs was done. Among the 7 second-line drugs used, E. coli was found to be most resistant to ceftazidime (86.5%) followed by cefotaxime (70.3%) and ofloxacin (51.4%), respectively. While imipenem was found more sensitive in second-line drugs with 91.9% sensitivity followed by amikacin (89.2%), amoxiclav (81.1%), and piperacillin/tazobactam (64.9%), respectively, as shown in Table 4.

Table 1: Distribution of bacterial isolates in total processed urine samples from children

| Gram (-ve) isolates     | Frequency (%) | Gram (+ve) isolates  | Frequency (%) |
|-------------------------|---------------|----------------------|---------------|
| Escherichia coli        | 66 (57.8)     | Staphylococcus aureus| 8 (7.0)       |
| Klebsiella pneumonia    | 18 (15.8)     | CoNS                 | 4 (3.5)       |
| Proteus spp.            | 10 (8.8)      |                      |               |
| Acinetobacter           | 4 (3.5)       |                      |               |
| Pseudomonas aeruginosa  | 2 (1.8)       |                      |               |
| Enterobacter spp.       | 2 (1.8)       |                      |               |
| Total                   | 102 (89.5)    | Total                | 12 (10.5)     |

MDR pattern in E. coli isolates
Most of the isolated E. coli pathogens showed resistance to more than 3 groups of antibiotics. It was found that 19.6% were sensitive to all antibiotics used in the study and 15.2% were resistant to 1 drug. 9.1%, 13.6%, and 42.4% isolates were resistant to 2, 3, and >3 drugs, respectively. Total MDR was found to be 37 (56.1%) out of 66 isolated samples which are shown in Table 5.

Different age and gender-wise distribution of MDR in E. coli
Table 6 shows the distribution pattern of MDR E. coli in male and female children of different age groups. From observations, it was found that 37 (56.10%) were found to be MDR E. coli out of a total of 66 E. coli.
positive samples. MDR strains were found more in females 23 (62.2%) than in males 14 (37.8%). Out of 14 multidrug-resistant E. coli isolated in males, the maximum number of isolates (n=9, 64.3%) was observed in age group 1–5 years, followed by age group below 1 year (n=4, 28.6%) and 5–10 year (n=1, 7.1%), respectively. While in females, maximum number of isolates (n=8, 34.8%) was observed in age group 1–5 years, followed by age group below 1 year (n=7, 30.4%), 10–15 year (n=6, 26.1%), and 5–10 year (n=2, 8.7%), respectively. This result was statistically insignificant (p>0.05).

**Distribution of MDR of *E. coli* in different hospital departments**

MDR of *E. coli* comprised of 37 (56.1%) samples out of a total of 66 *E. coli*. Culture positive cases for indoor and outdoor patients were 12 (32.4%) and 25 (67.6%), respectively, which are presented in Table 7.

UTIs are among the most common infection encountered in medical practices, causing major associated morbidity occurring from neonates to elderly people. The study demonstrated the valuable data to compare and monitor the status of variation in etiologic characteristics of UTI and their resistance patterns to antibiotics among uropathogens to improve efficient empirical treatment.

### Table 2: Distribution of *Escherichia coli* in different children age and gender group

| Age group in years | Positive no of *Escherichia coli* | Male | Female |
|--------------------|----------------------------------|------|--------|
| No. | % | No. | % | No. | % | No. | % |
| <1 | 15 | 22.7 | 6 | 6 | 9 | 22.5 | |
| 1–5 | 28 | 42.5 | 15 | 15 | 13 | 32.5 | |
| 5–10 | 12 | 18.2 | 4 | 4 | 8 | 20.0 | |
| 10–15 | 9 | 13.6 | 1 | 1 | 8 | 20.0 | |
| 15–18 | 2 | 3.0 | 0 | 0 | 2 | 5.0 | |
| Total | 66 | 100 | 26 | 100 | 40 | 100 | |

### Table 3: Distribution of *Escherichia coli* in different hospital departments

| Departments | Total sample cases | Positive cases | In percent (%) |
|-------------|--------------------|----------------|----------------|
| Outpatient  | 375                | 34             | 51.5           |
| Inpatient   | 155                | 32             | 40.5           |
| Total       | 530                | 66             | 100            |

### Table 4: Antibiotic susceptibility pattern of *Escherichia coli* among first- and second-line drugs

| First-line drugs | Sensitivity | Resistant | Second line Drugs | Sensitivity | Resistant |
|------------------|-------------|-----------|-------------------|-------------|-----------|
|                   | No. (%)     | No. (%)   |                   | No. (%)     | No. (%)   |
| Ampicillin       | 23          | 34.8      | 43                | 65.2        | Amikacin  | 33        |
| Ciprofloxacin    | 36          | 54.5      | 30                | 45.5        | Imipenem  | 34        |
| Ceftriaxone      | 4           | 6.0       | 62                | 94.0        | Ofloxacin  | 18        |
| Cotrimoxazole    | 24          | 36.4      | 42                | 63.6        | Tazobactam | 24        |
| Gentamicin       | 22          | 33.3      | 44                | 66.7        | Gatifloxine| 11        |
| Nitrofurantoin   | 51          | 77.3      | 15                | 22.7        | Gatiflozime| 5         |
| Norfloxacin      | 45          | 68.2      | 21                | 31.8        | Amoxiclav  | 30        |

### Table 5: Multidrug-resistance pattern in *Escherichia coli* isolates

| Organism        | Total | Resistant to |
|-----------------|-------|--------------|
| *Escherichia coli* | 66    | 1 Drug       |
|                 |       | 2 Drug       |
|                 |       | 3 Drug       |
|                 |       | >3 Drug      |
|                 | 10    | %            | 6              | 9.1        |
|                 | 15.2  | %            | 13.6           | 28         |
| Total MDR=37 (56.1%) |      | No        | 42.5          |            |
patients and hospitalized, which can be considered as the first-line therapy.

The highest percent of resistance toward second-line antibiotics was found for cefotaxime (86.5%) followed by cefotaxime (70.3%) and ofloxacin (51.4%), respectively, shown in Table 5. This finding is in agreement with the study conducted by Achariya et al. [27]. Imipenem was found to be the most effective drug against E. coli with susceptibility of 91.5% followed by amikacin with susceptibility of 89.2%. This finding was similar to the study conducted by Noor et al. [28]. The antimicrobial sensitivity pattern differs in different studies as well as different times even in the same hospital, because of the emergency of resistance strain as a result of indiscriminate use of antibiotics [29].

In this study, out of 66 positive isolates of E. coli, 37 (56.1%) were found to be MDR for E. coli. The high level of MDR observed in E. coli is due mediated by β-lactamase, which hydrolyze the β-lactam ring inactivating the antibiotics. The classical Team-1, Team-2, and SHV-1 enzymes are the predominant plasmid-mediated β-lactamase of Gram-negative rods [30].

CONCLUSION

The high prevalence rate of UTI in our study concludes E. coli as the predominant pathogen in children. The study showed that E. coli 66 (57.8%) was the major organism in UTI followed by K. pneumoniae 18 (15.8%) while other organisms were less dominant. Imipenem and Amikacin can be used as drugs (second line) of choice for multi-drug resistant (MDR) for E. coli. However, nitrofurantoin (first-line drug) can be used to treat UTI caused by E. coli. The high prevalence rate of UTI in our study concludes E. coli as the predominant pathogen in children. The finding of the study also demonstrated the fact that females were more susceptible to UTI than males may be due to anatomical proximity of the urinary system in females including sanitation and menstruation.

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AUTHORS’ CONTRIBUTIONS

All authors have almost equal contributions in this work as well as in the manuscript preparation.

| Age group in year | Positive no. of MDR of Escherichia coli | Male | Female |
|-------------------|----------------------------------------|------|--------|
|                   | No.  | %   | No.  | %   | No.  | %   |
| <1                | 11   | 29.7| 4    | 23.1| 7    | 30.4|
| 1–5               | 17   | 46.0| 9    | 57.7| 8    | 34.8|
| 5–10              | 3    | 8.1 | 1    | 15.4| 2    | 8.7 |
| 10–15             | 6    | 16.2| -    | -   | 6    | 26.1|
| 15–18             | -    | -   | 1    | -   | -    | -   |
| Total             | 37   | 100 | 14   | 100 | 23   | 100 |

| Departments | Positive no. of isolates | Percentage |
|-------------|--------------------------|------------|
| Outpatient  | 25                       | 67.6       |
| Inpatient   | 12                       | 32.4       |
| Total       | 37                       | 100        |
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