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Research Article

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

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ABSTRACT

Objective: This work aimed to detect the antibiotic susceptibility pattern of *Escherichia coli* isolated from children, as it is the most predominant pathogen of urinary tract infection (UTI).

Methods: About 530 urine samples were collected and tested using the modified Kirby–Bauer disk diffusion method to find the susceptibility pattern of isolated bacteria.

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 ceftazidime 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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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.

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

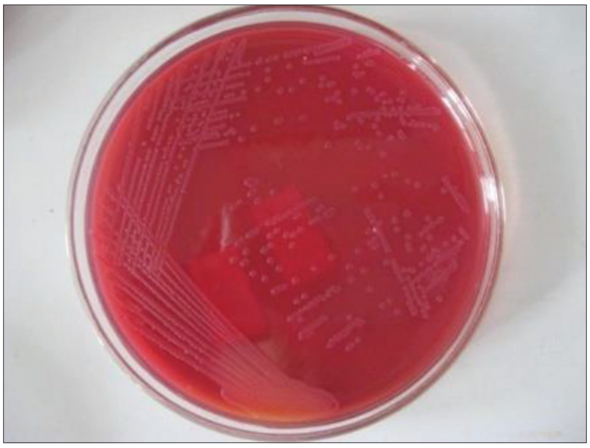


Fig. 1: Photograph of a pure culture of *Escherichia coli* in blood agar

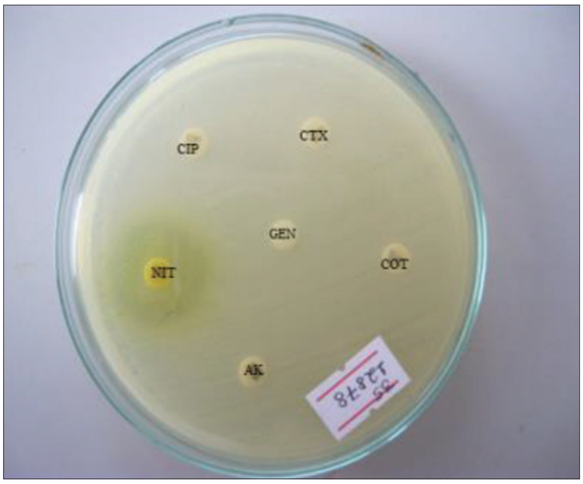
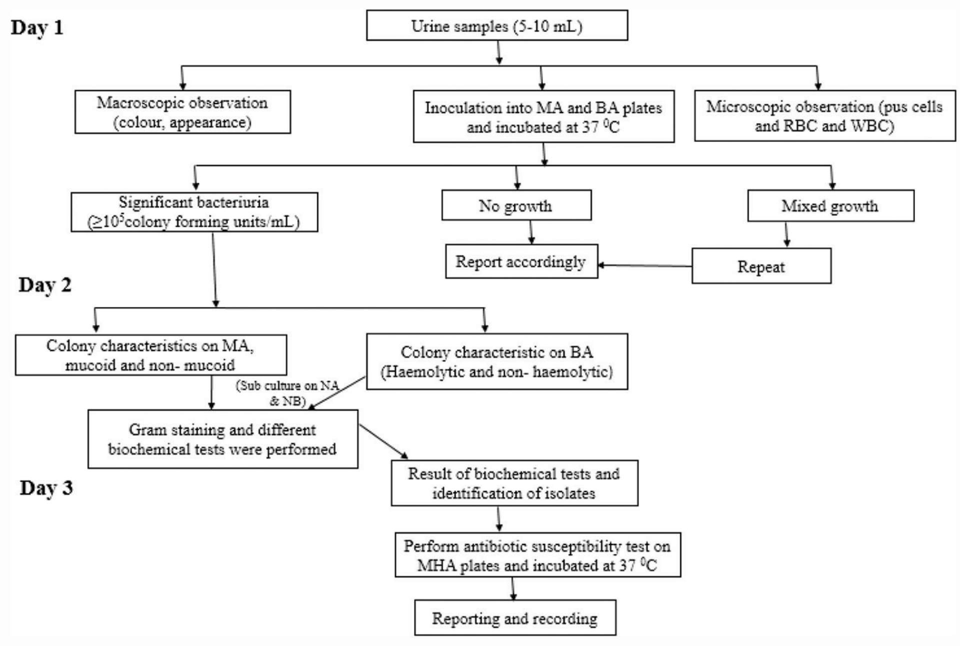


Fig. 2: Photograph of multidrug-resistant of *Escherichia coli* with (GEN: Gentamicin, AK: Amikacin, NIT: Nitrofurantoin, CIP: Ciprofloxacin, COT: Cotrimoxazole, CTX: Cefotaxime), respectively



Scheme 1: Flowchart representing the identification of UTI from urine samples

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 year group 1–5 years, followed by age group below 1 year (n=9, 22.5%), 5–10 year (n=8, 20%), 10-15 (n=8, 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.

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*

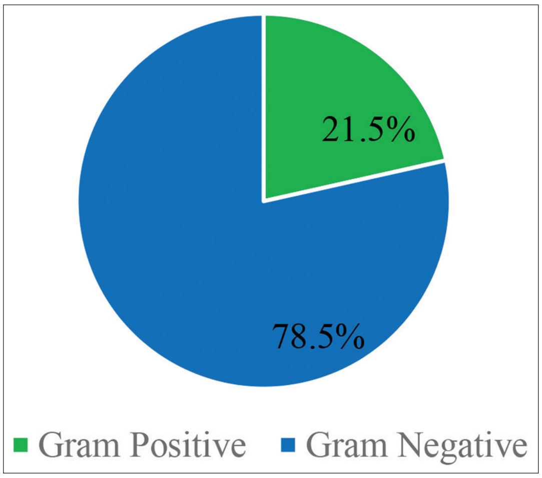


Fig. 3: Bacterial growth patterns in a total urine sample collected from children

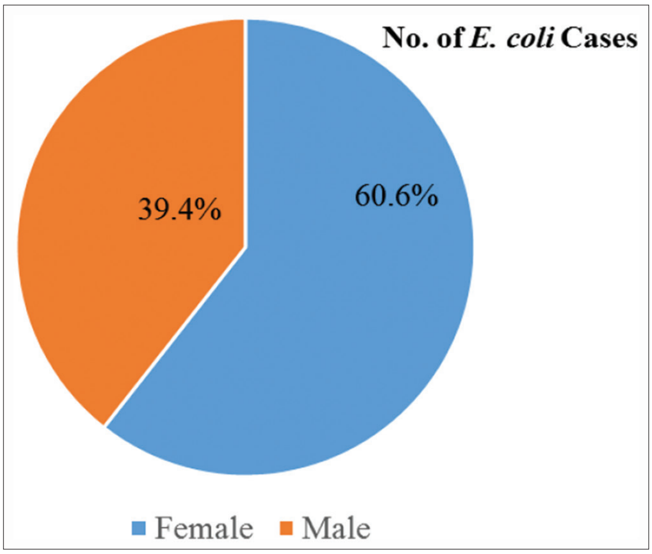


Fig. 4: Gender-wise distribution pattern of *Escherichia coli* in different urine samples of children

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)

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.	%
<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	48.5
Total	530	66	100

Among 530 collected urine samples, 416 (78.50%) samples showed no growth, and 114 (21.50%) samples showed significant growth. Among eight different species of bacteria isolated as major bacteria in urine samples, in the case of Gram-negative, *E. coli* 66 (57.8%) was found to be the most predominant organism followed by *Klebsiella pneumonia* 18 (15.8%), *Proteus* spp. 10 (8.8%), *S. aureus* 8 (7.0%), *Acinetobacter* spp. 4 (3.5%), *CoNS* 4 (3.5%), *Enterobacter* spp. 2 (1.8%), and *Pseudomonas aeruginosa* 2 (1.8%), as shown in Table 1. This result is also in harmony with the study done at international context [13] 84%, [14] 68.2%, [15] 80.0%, [16] 52.1%, [17] 76.7%, and [18] 49.3% [19], also found the predominance of Gram-negative in urine samples [20], states Gram-negative bacteria constituted the major group of isolates comprising 80.0%. The study revealed the colonic bacterial flora of the Enterobacteriaceae family is recognized to produce high UTIs, through the main pathway of canaliculi [21] in children.

Among the 114 (21.5%) growth positive samples in children, 33 (20.1%) were from males and 131 (79.9%) were from females. The male to female infection ratio of 1:4 was diagnosed to have UTI. Research studies performed by Akram *et al*. [22], also found the male to female ratio of UTI as 1:2.9 and [23] as 1:2. Many research experiments found that UTI cases in male children are higher than that of female but still the UTI cases are found more severe in female children in Nepal.

Further, in this study, the highest number of growth was observed in the age group between 1 and 5 years as 13 (32.5%) in female followed by age group below 1 year as 9 (22.5%), 5–10 year as 8 (20.0%), 10–15 year as 8 (20.0%), and 15–18 years as 2 (5.0%), respectively, as shown in Table 6.

In the present study, a total of 530 suspected urinary tract infected sample were processed, among them, 375 (70.8%) samples were from outdoor patients while 155 (29.2%) accounted for the indoor patient resulting high incidence of UTI among outpatient 51.5% than inpatient 48.5% as presented in Table 3. Similar findings were observed by the study done by Jesse *et al*. [24]. The proximity of the anus and the short urethral opening in females along with their hygienic habits are highly prone to UTIs [25].

Among the Gram-negative bacteria isolated, *E. coli* showed the highest percent of resistance toward first-line antibiotics, that is, ceftriaxone 94.0% and ceftazidime 86.5% as in Table 4. The highest percent of resistance toward first-line antibiotics was found for ceftriaxone (94.0%) followed by gentamicin (66.7%), and ampicillin (65.2%), respectively. According to Sharma *et al*. [26], *E. coli* is highly resistant to ampicillin (71.2%) in infants which are also similar to this study. Nitrofurantoin has shown an overall low resistance rate in both out-

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	89.2	4	10.8
Ciprofloxacin	36	54.5	30	45.5	Imipenem	34	91.9	3	8.1
Ceftriaxone	4	6.0	62	94.0	Ofloxacin	18	48.6	19	51.4
Cotrimoxazole	24	36.4	42	63.6	Tazobactam	24	64.9	13	35.1
Gentamicin	22	33.3	44	66.7	Cefotaxime	11	29.7	26	70.3
Nitrofurantoin	51	77.3	15	22.7	Ceftazidime	5	13.5	32	86.5
Norfloxacin	45	68.2	21	31.8	Amoxiclav	30	81.1	7	18.9

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

Organism	Total	Resistant to							
Escherichia coli	66	1 Drug		2 Drug		3 Drug		>3 Drug	
		No	%	No	%	No	%	No	%
		10	15.2	6	9.1	9	13.6	28	42.5
	Total MDR=37 (56.1%)								

Table 6: Different age and gender-wise distribution of MDR of *Escherichia coli* in patients

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	-	-	-	-	-	-
Total	37	100	14	100	23	100

Table 7: Frequency distribution of multidrug-resistant of *Escherichia coli* in different hospital departments

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

patients and hospitalized, which can be considered as the first-line therapy.

The highest percent of resistance toward second-line antibiotics was found for ceftazidime (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 Acharya *et al*. [27]. Imipenem was found to be the most effective drug against *E. coli* with susceptibility of 91.9% 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.

CONFLICTS OF INTEREST

No conflicts of interest are declared by the authors.

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