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Original article

Prevalence and Characterization of Multi-drug Resistant Uropathogens from Children with Urinary Tract Infections in Children Emergency Unit of Federal Teaching Hospital, Abakaliki (FETHA), Nigeria

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ABSTRACT

Objective: This study was designed to determine the prevalence of common etiological agents causing urinary tract infections in children emergency unit of FETHA and their susceptibility to antibiotics. **Materials and methods**: Eighty urine samples from both children's outpatient clinic and emergency wards of FETHA were collected using sterile universal containers. The collected samples were characterized using standard microbiology techniques. Isolated bacterial pathogens were subjected to antibiotic susceptibility test using the disc diffusion method. **Results**: Results showed that 74 bacterial isolates were obtained from the 80 urine samples collected. Thirty (37.5 %) isolates were *E. coli*, 12 (15.0 %) were *Pseudomonas aeruginosa*, 11 (13.75 %) were *Proteus* spp, 10 (12.5 %) were *Staphylococcus aureus*, 10 (12.5 %) were *Klebsiella* spp while 1(1.25 %) was *Enterococcus feacalis*. Susceptibility test results using some selected antibiotics against isolated bacterial pathogens showed that Imipenem was the least resisted antibiotic. *Klebsiella* spp exhibited the highest antibiotic resistance frequency as it was 100 % resistant to 9 out of 18 antibiotics tested. *E. coli* showed the highest multiple antibiotic resistance index (MARI) with a value of 24.45 while *S. aureus*, *Proteus* spp, *Klebsiella* spp and *P. aeruginosa* had MARI values of 6.97, 9.51, 8.90 and 9.03 respectively. **Conclusion**: This study showed that there is a high prevalence of multi-drug resistant uropathogens especially *E. coli*, among children with urinary tract infections in FETHA and the antibiotic sensitivity results showed that imipenem was the most active antibiotic against all the isolated pathogens.

KEYWORDS: Multi-drug resistant, uropathogens, children, antibiotics, bacterial isolates

INTRODUCTION

Urinary tract infection (UTI), also referred to as acute cystitis or bladder infection, is an infection that affects part of the urinary tract. When it affects the lower urinary tract, it is known as a simple cystitis (a bladder infection) and when it is associated with the upper urinary tract, it is known as pyelonephritis (a kidney infection). Bacterial UTIs can also involve the urethra, prostate, bladder or kidneys [1]. Urinary tract infections are found to be the common bacterial infections in children. It is hypothesized that UTI is caused by an ascending infection via the urethra.

Colonic bacteria, implicated especially Enterobacteriaceae, are the commonest organisms isolated from children with uncomplicated UTI. In 75 % to 90 % of female children with UTI, the incriminating organism is usually *Escherichia coli* followed by *Klebsiella* and *Proteus* while in males, *Proteus* is as common as *E. coli* as a bacterial cause of UTI [2].

The percentage prevalence of UTI in girls is about 3 % to 5 % and 1 % in boys during childhood, with the first attack occurring in girls by 5 years, peaking during infancy, and toilet training, where as it is more common in boys during the first year of life, especially among those who are uncircumcised [3]. The Male: Female ratio in UTI varies with age, observed as 2.8 – 5.4: 1.0 in first year of life [4]. The diagnosis of UTI is very often missed in young children; this is as a result of minimal and nonspecific symptoms. The increasing developing renal cortex in young children is vulnerable to renal scarring resulting in hypertension and chronic renal failure. These high morbidities in adults often have their origin in childhood [5].

A clinically suspected case of UTI should be diagnosed in a standard laboratory and antimicrobial susceptibility patterns are defined and documented with urine culture report. After the diagnosis of UTI, its category should be defined. This helps in guiding a clinician about the appropriate radio/nuclear imaging evaluation, choice of antimicrobial agent, duration of treatment and need of chemoprophylaxis [5]. Antimicrobial agents have been the only easily and widely used therapeutic choice available to oppose the infections caused by urinary tract pathogens. Microbial populations have developed various strategies to combat these microbial agents: a major contributing factor in the development of antimicrobial resistance worldwide [6].

With the growing number of emerging uropathogens and the simultaneous increase of newer antibiotics, it is mandatory that laboratories use standardized methods and report only appropriate antibiotics for UTIs. Historically, one of the primary functions of the Clinical Microbiology Laboratory has been to measure antimicrobial susceptibility patterns. Early diagnosis of UTI in young children is important to prevent urinary tract abnormalities, to preserve renal function of the growing kidney [2]. This study investigates the prevalence and antibiotic sensitivity patterns of common etiological agents causing urinary tract infections in children emergency unit of FETHA.

MATERIALS AND METHODS

Sample Collection

A total of 80 urine samples from both children's outpatient clinic and children's Emergency Ward of Federal Teaching Hospital Abakaliki including; mid-stream urine and catheter tips were collected with labeled sterile universal container. After collection, samples were immediately transported to Applied Microbiology Laboratory Unit of Ebonyi State University, Abakaliki, for bacteriological analysis. The samples were analyzed within 45 minutes of collection.

Bacteriological Analysis of Urine samples: Each urine sample previously inoculated on nutrient broth which showed turbidity/microbial growth was re-inoculated after 18-24 hours onto MacConekey agar, Cystine lactose electrolyte deficiency agar (C.L.E.D.) plates and incubated aerobically at 37 ^{o}C for 18 – 48 hours. After incubation, bacterial growths were observed for colony appearance and were Gram stained and subjected to further physiological and biochemical tests such as catalase test, motility test and other biochemical tests such as coagulase test, oxidase test, citrate test, indole test, Methyl red Voges-Proskauer (MRVP) test, urease test, hydrogen sulphide production test, and sugar fermentation test [7]. API kit was used to confirm the organisms.

Antibiotics Susceptibility Test (Disc Diffusion Technique)

A sterile swab stick was used to inoculate the pure culture of the organism on the plate of Mueller-Hinton agar medium. The surface of the medium was streaked in four different directions while the plate was rotated approximately 60^{o} to ensure even distribution. With the Petri dish lid in place, the surface of the Mueller-Hinton agar medium was allowed to dry for 25 minutes. A sterilized forceps was used to place the antibiotic discs on the inoculated Mueller- Hinton agar (evenly distributed) so that the disc will be about 15 mm from the edge of the plate and not closer than 25 mm from disc to disc. After 30 minutes, the plates were inverted and incubated for 24 hours.

A metre ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate [7]. The result was interpreted as 'sensitive' or 'resistant' based on the diameters of zones of inhibition of bacterial growth as recommended by Clinical and Laboratory Standards Institute [8]. The following standard antibiotic discs (Oxoid, UK) were used against the isolates: Ceftriaxone (CRO, 30ug), Penicillin (P, 10 ug), Gentamicin (CN, 30ug), Amoxycillin (AML, 25ug), Nitrofurantoin(F, 300 ug), Tetracycline (TE, 10ug), Amoxycillin/Clavulanic acid (AMC, 30 ug), Trimethoprim–Sulphamethoxazole (SXT, 25 ug), Ofloxacin (OFX, 5 ug), Cefotaxine (CFX, 30 ug), Tobramycin (TOB, 30 ug), Ceftazidime (CAZ, 30 ug), Imipenem (IPM, 10 ug), Ertapenem (ETP, 10 ug) and Nalidixic acid (NA, 30 ug).

Multiple Antibiotic Resistance Index (MARI)

Multiple antibiotic resistance Index (MARI) was determined to ascertain the resistance level of the isolates: that is, the number of antibiotics to which the test isolates exhibited resistance.

MARI = a/b.

Where a = number of antibiotics to which the isolate exhibited resistance; b = total number of antibiotics to which the isolate was subjected to [9].

RESULTS

Seventy four bacterial pathogens were isolated from the eighty (80) urine samples collected (Table 1). Out of the 74 bacterial pathogens isolated, 10 (13. 5 %) were *Klebsiella* spp., 30 (40.5 %) were *E. coli*, 10 (13.5 %) were *S. aureus*, 12 (16.2 %) were *P. aeruginosa*, 11 (14.9) were *Proteus* spp. while 1 (1.35 %) was *Enterococcus feacalis*. *E coli* 30 (40.5%) was the leading etiologic agent of pediatric UTI in FETHA; followed by *Pseudomonas auruginosa* 12(16.2 %), *Proteus* spp 11(14.9 %), *Klebsiella* spp 10(13.5 %), *S. aureus* 10(13.5 %) and *Enterococcus feacalis* 1(1.35%) being the least (Table 1).

Table 1: Frequency of bacterial pathogens isolation from children with UTI in FETHA

Total number of samples collected/examined	Total number of bacterial pathogens isolated	Number and type of bacterial pathogens isolated (%)
80 Urine samples	74	Klebsiella spp. 10 (13.5)
		E. coli 30 (40.5)
		S. aureus 10 (13.5)
		P. aeruginosa 12(16.2)
		Proteus spp 11 (14.9)
		Enterococcus feacalis 1 (1.35)

Table 2: Rate of bacterial pathogen isolation among children with UTI in FETHA

SEX	MALE		FEMALE		TOTAL
Age	0-12 months	1-12 years	0-12 months	1-12 years	
Organism isolated					
E. coli	4 (30.8 %)	10 (55.6 %)	10 (45.5 %)	6 (27.3 %)	30
S. aureus	2 (15.4 %)	2 (11.1 %)	2 (9.1 %)	4 (18.2 %)	10
Pseudomonas species	2 (15.4 %)	4 (22.2 %)	4 (18.2 %)	4 (18.2 %)	12
Enterococcus species	0 (0 %)	0 (0 %)	1 (4.5 %)	0 (0 %)	1
Proteus species	2 (15.4 %)	2 (11.1 %)	3 (13.6 %)	4 (18.2 %)	11
Klebsiella species	2 (15.4 %)	2 (11.1 %)	2 (9.1 %)	4 (18.2 %)	10
Total	13 (100 %)	18 (100 %)	22 (100 %)	22 (100 %)	75

Table 2 shows the rate of bacterial pathogen isolation among children with UTI in FETHA. The age bracket for males ranging from 0-12 months had 13 (17.3 %) bacterial pathogens; 1-12years had 18 (24.0 %), while for females, 0-12 months had 22 (29.3 %) and 1-12 years had 22 (29.3 %) (Table 2).

All the isolates were resistant to more than two classes of antibiotics. The antibiogram results showed that *E. coli* was 100 % resistant to Nalidixic acid (Tables 3 and 5). *E. coli* had the highest Multiple Antibiotic Resistance Index (MARI) value (a total of 24.45) while *S. aureus*, *Proteus* spp, *Klebsiella* spp and *P. aeruginosa* had 6.97, 9.51, 8.9 and 9.03 respectively (Table 4). *S. aureus* had the least MARI value (6.97). *Klebsiella* spp. had a higher MARI (8.9) despite having the same incidence rate with *S. aureus* (Table 4). *Klebsiella* spp had the highest rate of resistance against 9 antibiotics (including nitrofurantoin, tetracycline, trimethoprim-sulfamethoxazole and nalidixic acid) (Table 5). *S. aureus*, *Klebsiella* spp and *Proteus* spp were 100 % resistance to ceftazidine (Table 5).

Four bacterial isolates (*E. coli*, *Staph aureus*, *Klebsiella* spp and *P. aerugnosa*) were resistant to penicillin (Table 5). The bacterial pathogens exhibited a resistance frequency of 90 % against nitrofurantoin (Table 5). Results also showed that the *E. coli* isolates were completely resistant (100 %) to nalidixic acid (Table 5). Almost all the *E. coli* isolates were resistant to all the antibiotics tested except imipenem which was found to be the most active agent (Table 5).

Table 3: Antibiotic resistance frequency of bacterial isolates

Antibiotics	*E. coli* resistance (%)	*P. aeruginosa* resistance (%)	*Proteus* spp resistance (%)	*S. aureus* resistance (%)
AMX	80	90	72	50
P	100	100	90	100
AMC	72	82	81	60

TE	95	100	100	90
CN	55	68	72	50
TOB	85	68	81	40
ETP	55	68	90	70
IPM	22	15	44	10
CAZ	95	82	100	100
CTX	90	90	100	60
CRO	92	82	100	80
F	90	74	100	80
OFX	63	65	72	60
NA	100	90	100	90
SXT	92	72	90	60

Key: AML= Amoxycillin, P= Penicillin, AMC= Amoxycillin/ Clavulanic acid, TE= Tetracycline, CN= Gentamicin, TOB= Tobramycin, ETP= Ertapenem, IPM= Imipenem, CAZ= Ceftazidime, CTX= Cefotaxime, CRO= Ceftriaxone, F= Nitrofurantoin, OFX= Ofloxacin, NA= Nalidixic acid, SXT= Sulfamethoxazole/ Trimethoprim, OX= Oxacillin, E= Erythromycin, DA= Clindamycin

Table 4: Multiple Antibiotic Resistance Index (MARI) of isolated bacterial pathogens from children with UTI in FETHA

30 *E. coli* isolates															
Isolates	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15
MARI value	0.66	0.53	0.93	0.80	0.60	0.66	1.00	0.86	0.80	0.80	0.93	0.93	0.93	0.86	0.80
Isolates	E16	E17	E18	E19	E20	E21	E22	E23	E24	E25	E26	E27	E28	E29	E30
MARI value	0.53	0.66	0.53	0.80	0.86	0.73	0.80	0.80	0.86	0.66	0.66	1.00	1.00	1.00	0.93
Total	**24.45**														
10 *S. aureus* isolates															
Isolates	1	2	3	4	5	6	7	8	9	10					
MARI value	0.61	0.51	0.77	0.88	0.83	0.55	0.83	0.55	0.72	0.72					
Total	**6.97**														
11 *Proteus* species isolates															
Isolates	1	2	3	4	5	6	7	8	9	10	11				
MARI value	0.66	0.93	0.60	0.86	0.60	0.86	1.00	1.00	1.00	1.00	1.00				
Total	**9.51**														
10 *Klebsiella* species isolates															
Isolates	1	2	3	4	5	6	7	8	9	10					
MARI value	0.93	0.86	0.86	0.66	1.00	0.66	0.93	1.00	1.00	1.00					
Total	**8.9**														
12 *Pseudomonas aeruginosa* isolates															
Isolates	1	2	3	4	5	6	7	8	9	10	11	12			
MARI value	0.33	0.66	0.66	1.00	1.00	0.93	0.93	0.66	0.80	0.80	0.46	0.80			
Total	**9.03**														

Table 5: Percentage (%) resistance frequency of bacterial pathogens to test antibiotics

Isolates	AML	P	AMC	TE	CN	TOB	ETP	IPM	CAZ
E. coli	24 (80)	30 (100)	22 (73.3)	29 (96.6)	17 (56.6)	26 (86.6)	17 (56.6)	7 (23.3)	29 (96.6)
Pseudo.	11 (91.6)	12 (100)	10 (83.3)	12 (100)	7 (58.3)	7 (58.3)	7 (58.3)	2 (16.6)	10 (83.3)
Staph.	5 (50)	10 (100)	6 (60)	9 (90)	5 (50)	4 (40)	7 (70)	1 (10)	10 (10)
Kleb.	10 (100)	10 (100)	10 (100)	10 (100)	6 (60)	10 (100)	8 (80)	4 (40)	10 (100)
Prot.	8 (72.7)	10 (90.9)	9 (81.8)	11 (100)	8 (72.7)	9 (81.8)	10 (90.9)	5 (45.5)	11 (100)

Isolates	CTX	CRO	F	OFX	NA	SXT	OX	E	DA
E. coli	27 (90.9)	28 (93.3)	27 (90.0)	19 (63.3)	30 (100)	28 (93.3)			
Pseudo.	11 (91.6)	10 (83.3)	9 (75)	8 (66.6)	11 (91.6)	9 (75)			
Staph.	6 (60)	8 (80)	8 (80)	6 (60)	9 (90)	6 (60)	7 (70)	8 (80)	9 (90)
Kleb.	10 (100)	8 (80)	10 (100)	9 (90)	9 (90)	10 (100)			
Prot.	11 (100)	11 (100)	11 (100)	8 (72.7)	11 (100)	10 (90.9)			

Key: *Pseudo* = *Pseudomonas aeruginosa*, *Staph.* = *Staphylococcus aureus*, *Kleb.* = *Klebsiella* species, *Prot. Proteus* species, AML=Amoxycillin, P = Penicillin, AMC = Amoxycillin/Clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO – Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulfamethoxazole/Trimethoprim OX = Oxacillin, E = Erythromycin, DA = Clindamycin.

DISCUSSION

Urinary tract infections (UTIs) are common bacterial infections in children. The diagnosis of UTI is very often missed in young children due to minimal and nonspecific symptoms. The developing renal cortex in young children is vulnerable to renal scarring resulting in hypertension and chronic renal failure [5].

This study shows the prevalence and antimicrobial sensitivity patterns of bacterial pathogens implicated in UTI from pediatric patients attending Federal Teaching Hospital Abakaliki (FETHA). *Escherichia coli* (40.5 %) was the most prevalent Gram-negative bacteria pathogen isolated. This result is in agreement with reports from other studies [10], [11]. The studies on UTI in children in other places of the world also showed that *E. coli* is the common pathogen causing UTI [12].

Gram-positive organisms have received more attention recently as a cause for bacteriuria and UTI in children. *S. aureus*, *Streptococci* and *Enterococcus faecalis* have been reported in small numbers by various researchers, but they are recognized as important causes of UTI [13]. Interestingly, we found similar occurrence rate of 13.5 % and 1.35 % for *S. aureus* and *Enterococcus feacalis* respectively. Higher incidence of Gram-negative bacteria, belonging to the Enterobacteriaceae; in causing UTI has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize the urogenital mucosa with adhesins, pilli, fimbriae and P – 1 blood group phenotype receptor [14].

In this study, the frequency of bacterial pathogens isolation from children with UTI in FETHA was also studied. *E coli* 30 (40.5 %) was the leading etiologic agent of pediatric UTI in FETHA, followed by *Pseudomonas aeruginosa* (16.2 %), *Proteus* spp (14.9 %), *Klebsiella* spp (13.5 %), *S. aureus* (13.5 %) and *Enterococcus feacalis* (1.35 %). Badhan *et al.* reported that *E. coli* (42.3 %) was the most common aetiologic agent of UTI in 192 paediatric patients in Punjah, India [15]. Bashir *et al*. and Kalsoom *et al.* also reported very high prevalence rates of *E. coli*; (66 %) and (98 %) respectively [16], [17]. Uropathogenic *Escherichia coli* (UPEU) is responsible for > 80 % of community – acquired UTIs with most other infections caused by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus faecalis* [18]. The source of UTI pathogens is generally considered to be the patient's own flora, preceded by colonization of the vagina and periurethral area by uropathogens from the gastrointestinal tract.

In this study, significant differences between age groups and ordering among years were observed just like Kiffer *et al.* reported in his study [19]. A total of 41.3 % bacterial pathogens were isolated from male patients while 58.7 % were from female. The age bracket for males ranging from 0-12 months had 13 (17.3 %) bacterial pathogens, 1-12 years had 18 (24.0 %) while for females, 0-12 months had 22 (29.3 %) and 1-12 years had 22 (29.3 %). This study is in agreement with the work of Sharifian *et al.* who reported that the male to female ratio was 1:2 [20]. In other studies, UTI occurred in the ratio 1:3 to 3 times as many males as females during the neonatal period. Gender-specific differences in the rates of UTI may depend on the age. Boys are more commonly infected during the first 3 months of life, and after the first year, symptomatic UTI is more frequent among girls. Similarly, asymptomatic bacteriuria is more frequently detected in boys than in girls during the first 12 months of life. Thereafter, the incidence decreases markedly in boys but increases in girls [21].

This study also agrees with Kiffer *et al.* observation that inappropriate and wide spread use of antibiotics, selective pressures and other multiple factors could contribute in hampering control measures towards increase in antimicrobial resistance today [19]. Handling pediatric patients in our healthcare system today is intellectually tasking especially with regards to administering drugs. Wrong dilutions could be a serious contributing factor as regards development of resistant strains in children.

Our study revealed that *E. coli* had the highest Multiple Antibiotic Resistance Index (MARI) value (a total of 24.45) while *S. aureus*, *Proteus* spp, *Klebsiella* spp and *P. aeruginosa* had 6.97, 9.51, 8.9 and 9.03 respectively. The very high MARI presented by *E. coli* could be as a result of their high incidence recorded in this study. *S. aureus* had the least MARI value (6.97). In this present study, all the isolates were resistant to at least two classes of antibiotics, thus depicting their multi-drug resistance traits. This result is in concord with the findings of Osundiya *et al.*, who reported that a third of the isolates were resistant to two classes of antibiotics [22]. *Klebsiella* showed MARI value of 8.9. This finding is in contrast to the findings of Osundiya *et al.* who reported MARI value of 0.4 for *Klebsiella* spp in their study [22].

This further shows the high resistance of these isolates to the antibiotics. In this study, antibiogram carried out on the isolated bacterial pathogens showed that *E. coli* was 100 % resistant to Nalidixic acid. This was not in agreement with the findings of Afsharpaiman *et al.* who reported that *E. coli* has the lowest resistance to nalidixic acid in children [23]. It agrees with the findings of Badhan *et al.* who found that nalidixic acid showed high resistance to all isolates especially the Gram-negative organisms [15]. *Klebsiella* spp had the highest rate of resistance against 9 antibiotics (including nitrofurantoin, tetracycline, trimetroprim-sulfame-thoxazole, nalidixic acid). This agrees with the findings of Kiffer *et al.* who also recorded a significant resistance rates to nitrofurantoin, tetracycline, trimethoprim-Sulfamethoxazole and nalidixic acid (21.2 %, 19.8 %, 17.75 % and 15.2 % respectively) [19]. *S. aureus*, *Klebsiella* spp and *Proteus* spp were 100 % resistant to ceftazidine. This agrees with the work of Osundiya *et al.* who recorded a very high level of resistance to ceftazidime 15(71.4 %) [22].

Other cephalosporins also demonstrated high levels of resistance. This study reveals that four of the bacterial species were resistant to penicillin. The isolates include: *E. coli*, *S. aureus*, *Klebsiella* spp and *P. aeruginosa*. These isolates also resisted tetracycline to a very high significant level. This reflected the fact that penicillin and tetracycline were the most commonly prescribed antibiotics in the hospital even before the results of urine culture analysis and also the readily available antibiotics in the market. The widespread use and more often the misuse of antimicrobial drugs has led to a general rise in the emergence of resistant bacteria [24].

In this study, imipenem was the most active antibiotic against all the isolated uropathogens. This agrees with the findings of Osundiya *et al.* who reported 94.15 % susceptibility of all the isolated pathogens to imipenem [22]. *E. coli* strains were virtually resistant to all the antibiotics except imipenem which was found to be the most active agent. The antibiotic resistance frequency of *E. coli* in this study disagrees with other studies. Modarres *et al.* found that *E. coli* was the most susceptible bacterial isolate to aminoglycosides and had the lowest resistance frequency to nalidixic acid [25]. In another study by Sharifian *et al.*, *E. coli* was shown to be most susceptible to ceftriaxone, ceftizoxime and cefotaxime [20].

Based on our findings, imipenem, ofloxacin and gentamycin are still appropriate for initial empirical intravenous therapy for UTI among children despite an increased resistance rate of *E. coli* isolates. Our study also found that the isolated bacterial pathogens had multi-drug resistance activity against nitrofurantoin with a resistance rate of 90.0 %. However, in previous studies, nitrofurantoin was found to be very active against Enterobacteriaceae. Some studies even recommended nitrofurantoin as the first choice among oral antibiotics for prophylaxis and treatment of UTI in children [26]. *Pseudomonas aeruginosa* strain was the only isolate that did not exhibit multi-drug resistance. This finding is in agreement with the report of Osundiya *et al.* [22].

CONCLUSION

This study showed that there is a high prevalence of multi-drug resistant uropathogens especially *E. coli*, *S. aureus*, *Proteus* spp, *Klebsiella* spp, and *Pseudomonas aeruginosa* among children with urinary tract infections in children emergency unit of FETHA. This study also established that imipenem was the most active antibiotic against all the isolated uropathogens unlike penicillin and ceftazidime in which high frequency of resistance were observed.

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