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Prevalence and Antimicrobial Susceptibility Profile of Uropathogens in Children Attending Tertiary Health Care Centre in Enugu, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author CUM conducted the study and drafted the manuscript. Author OAI designed and assisted in laboratory investigations. Author PIU wrote the protocol and assisted in the laboratory investigations. Author NIO managed the literature search. Author EAO managed the analysis of the study. Author EMO prepared and arranged the manuscripts. All the authors read and approved the final manuscript.

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ABSTRACT

Infections of the urinary tract are one of the most prevalent bacterial infections in developing countries. It is a challenge that is most often encountered in pediatrics. Uncontrolled use of antimicrobial drugs has led to the development of drug-resistant organisms. Hence, this study was aimed at isolating, characterizing, and identifying the pathogens associated with urinary tract infection (UTI) in children attending Tertiary Health Care Centre in Enugu metropolis and to determine their antimicrobial susceptibility profiles. Urine samples were collected from 260 patients with signs and symptoms suggestive of urinary tract infection (UTI). The children were seen in children out-patients, children emergency departments of the hospital, and pediatric ward. Their ages ranged from 0-17 years. The urine samples were analyzed in the laboratory using standard

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bacteriological methods. Antibiotic sensitivity of the isolates was determined by the Kirby-Bauer disc diffusion technique. Of the 260 urine samples, significant bacterial agents were recorded in 98(37.7%). *Escherichia coli* ranked highest with 30(32.6%) followed by *Klebsiella spp*. 17(18.5%) and the least was *Enterococcus fecalis* 1(1.1%). Gender distribution showed a higher prevalence in females ($p < 0.05$). The highest positive result was obtained from children between 0-5years. Most of the isolates were sensitive to Imipenem, Nitrofurantoin, Ceftriaxone, and Ciprofloxacin but resistant to Augmentin and Amoxicillin. There is a need to regularly monitor the antimicrobial susceptibility profile of these etiological agents to keep track of the effectiveness of certain therapeutic agents.

Keywords: Urinary tract infections; children; etiology; antimicrobial susceptibility; Nigeria.

1. INTRODUTION

Infections of the urinary tract (UTI) are mostly encountered in pediatrics [1,2]. It is a challenge that pediatric health care providers often face, whereas UTIs do not occur as often in children than they do in adults, they can be an important source of morbidity in children [1,3]. Urinary tract infection (UTI) is the presence of pathogens undergoing multiplication anywhere along the urinary tract [4]. These pathogens could be bacteria, fungi, parasites or viruses. There are three broad divisions of infections of the urinary tract (UTI) namely, pyelonephritis which is infection of kidney or its parts by bacteria (upper UTI), cystitis, which is defined as the inflammation of the bladder (lower UTI) and asymptomatic bacteriuria which is the presence of bacteria in urine, yet no clinical symptoms of UTI. The feverish infant or child that has significant bacteriuria in addition to abdominal or flank pain, malaise, nausea, vomiting has pyelonephritis [5,6]. Urine is sterile under normal conditions of good health. Significant bacteriuria is growth with a colony count of $\geq$ 100,000 CFU/ml of clean catch mid-stream urine [7,8].

Nearly all UTIs are ascending in origin [1]. Throughout infancy, the symptoms and signs of infections of urinary tract are non specific. Most prevalent symptoms of infections of urinary tract in the first two years of life are fever of unknown origin. The signs and symptoms after the second year of life are fever, chills, rigor, and flank pain. Suprapubic discomfort, dysuria, frequent urination, urgency, blurred urine, foul-smelling urine, and suprapubic tenderness are indicators of lower tract [9]. Most infections of the urinary tract start from the bladder and then the pathogens spread to the kidneys (pyelonephritis) and may proceed to the bloodstream. Most experiences of the infection of the urinary tract (UTI) in the first year of life are pyelonephritis [10]. Cystitis which is simple may ordinarily advance to pyelonephritis. Despite evidence of genetical predilection, patients likely to have pyelonephritis remain unpredictable [1]. The etiological agent varies from region to region. Most of the infections of the urinary tract are caused by gram-negative bacteria like *Escherichia coli*, *Proteus spp, Klebsiella spp, Pseudomonas aeruginosa*, *Acinetobacter, Serratia* and *Morganella marganii.* Gram-positive organisms may also cause UTI namely *Enterococcus*, *Staphylococcus* especially coagulase negative *Staphylococci* and *Streptococcus agalactiae* [11,12]. *Escherichia coli* are the most prevalent gram-negative species in young girls, while *Proteus species* predominate in boys [13]. Epidemiology of UTI in infancy differs by age, gender, and other factors. UTIs occur in 5% of girls and 1 to 2% of boys. In the first one year of life in all children, highest prevalence of UTI is seen in 1% and approximately 0.1-1.0% in all newborn infants to as high as 10% in low birth weight infants. Before age one, infections of urinary tract are found more in boys than girls. After age one, Urinary tract infections are commonly seen in girls [5,13]. Children receiving broad-spectrum antibiotics such as cephalexin and amoxicillin that are known to change the gastrointestinal and periurethral flora are at high risk for UTI [1]. Diagnosis of UTI requires a correctly collected urine [14]. Any number of colonies from suprapubic bladder that has more than 10^3 colonies from intermittent catheterization and more than 10^5 colonies per millilitre from a sample of midstream clean-catch urine shows UTI. Most infections of the urinary tract come from a single organism. Two or more organisms are usually found to indicate contamination [15, 16].

In Nigeria, like other developing countries of the world, there is a high prevalence of bacterial infections and treatment of these infections is often very poor, some even resort to traditional/

herbal medications. Most cases of uncomplicated UTIs can be mild and transient, but the after-effect of untreated UTIs can be very serious hence accurate and timely diagnosis of UTI is very crucial [1].

Indiscriminate use of antimicrobial agents and other related factors have led to the appearance of antimicrobial-resistant bacteria organisms [17]. Treatment options are narrowing down due to drug resistance; in most cases antibiotics treatment is usually started empirically before urine culture results are available, knowledge of the etiological agents and their susceptibility profile are necessary to ensure appropriate treatment. Updating of antibiotics susceptibility profile is paramount for the proper management of patients in our environment.

2. MATERIALS AND METHODS

2.1 Study Design

This was a hospital-based cross-sectional study done in the bacteriology unit of Microbiology laboratory at the University of Nigeria Teaching Hospital (UNTH), Ituku/Ozalla, Enugu from January – November 2018. They were children suspected of having urinary tract infections from Children outpatients department, children emergency department, and pediatric ward.

2.2 Inclusion Criteria

Children between the ages of 0 to 17 years being suspected of having UTI and presenting with signs and symptoms of urinary tract infection and who had not taken antibiotics in the last 14 days were included in the study.

2.3 Exclusion Criteria

Children whose parents did not give their consent and who had taken antibiotics were excluded from the study.

2.4 Specimen Collection

Sample size was determined using formula of Kish Leslie (1965): $n = Z^2 p\ (1\text{-}p)/\ d^2$, where z= Z score for 95% confidence interval (CI) = 1.96, p= prevalence and d= acceptable error (5%). We used the prevalence of obtained by Ibeneme et al. in Enugu which was 11% [18]. This gave the minimum sample of 151 however, due to large patient numbers available we enrolled 260 children. We used clean-catch mid-stream urine in most patients and catheter aspirated in some patients. The parent/guardian was instructed on how to collect sample. In case of catheterized patients, urine samples were collected either through the catheter collection port or through puncture of the tube with a sterile needle by the nurse on duty in the presence of the researcher. Urine samples were collected from 260 children between the ages of 0-17years in a sterile screw-capped universal container or sterile needle, well labelled with the date, age and sex of the patient. The samples were immediately processed without delay.

2.5 Laboratory Diagnostic Methods

Specimens were carefully cultured on freshly prepared and dried blood agar and Cysteine Lactose Electrolyte Deficient Agar (CLED) using a standard wire-loop. The plates were incubated at 37°C for 24hr and a CFU count of 10^5/ml of urine was considered significant for UTI. The identification of isolates was done by conventional methods including colony morphology, gram stain, motility tests, and biochemical tests [19].

2.6 Antimicrobial Susceptibility Tests

Antimicrobial susceptibility tests and interpretations for the isolated organisms were done by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Himedia) following the zone size criteria as per standard guidelines. The diameters of the zones of inhibition were measured accordingly [20,21].

The following antimicrobials were used ofloxacin (5µg), Amoxicillin (30 µg), Ciprofloxacin (5 µg) Nitrofurantoin (300 µg). Gentamycin (10 µg), Imipenem (10 µg) Ceftazidime (30 µg), Cefuroxime (30 µg), Azithromycin (15 µg), Levofloxacin (5 µg), and Augmentin (30 µg).

3. RESULTS

Of the 260 urine samples analyzed, 98 (37.7%) isolates were obtained. *Escherichia coli* ranked highest 30(32.6%), others were *Klebsiella* spp.17 (18.5%), *Citrobacter* and *Staphylococcus aureus* 12(13%) respectively*, Proteus* 11(12%), *Pseudomonas* 9(9.8%), *Enterobacter* 6(6.5%) and *Strep.spp* 1(1.1%) (Table 1).

Table 2 shows the distribution of isolates according to age and gender. Of the 260 samples examined, females had the highest number of positive cases with a prevalence of

68(26.2%) while males had 30(11.5%) and this was statistically significant (P< 0.05). Age-wise distribution showed that 0-5 year ranked highest 38(39.2%), followed by 16 -17 years, 32(33%), the least was in 6-10 years with a prevalence of 12(12%).

Table 3 shows the antimicrobial susceptibility pattern of gram-negative organisms, Antimicrobial susceptibility test showed that all isolates of *Escherichia coli* were 100% sensitive to Imipenem, Nitrofurantoin 26(86.7%), Ceftriaxone 22(73.3%), Levofloxacin 20(66.7%) and Ciprofloxacin 18(60%) but were 100% resistant to Amoxicillin, Augmentin 29(96.7%), Cefuroxime 22(73.3%), Gentamycin 18(60%).

Klebsiella spp isolates were 100% sensitive to Imipenem and Nitrofurantoin and Ofloxacin 12(70.6%) respectively. *Proteus* was sensitive to Imipenem 11(100%), Ceftriaxone 8(72.7%), Nitrofurantoin 7(63.3%), Levofloxacin 5 (54. 5%), but 100% resistant to Azithromycin and Cefuroxime. *Enterobacter* was 100% sensitive to Imipenem and Cefuroxime, but 100% resistant to levofloxacin and Azithromycin.

Pseudomonas aeuroginosa isolates were sensitive to Imipenem 9(100%), Ciprofloxacin 8(88.9%), Nitrofurantoin 7(77.8%), but 100% resistant to Amoxicillin and Ceftriaxone.

Most isolates of *Citrobacter* were sensitive to Imipenem 10(83.3%), Nitrofurantoin 9(75%), Ciprofloxacin 7(58.3%), but resistance to Augmentin 12(100%), Amoxicillin 10(83.3%), Ofloxacin, Ceftazidime and Azithromycin 9 (75%).

Table 4 shows the antimicrobial susceptibility profile of gram-postive organisms.

All isolates of Staphylococcus aureus were sensitive to Imipenem 12(100%), Ciprofloxacin 10(83.3%), Nitrofurantoin 8(66.7%), Levofloxacin 6(50%) but 100% resistant to Cefuroxime and Azithromycin.

Enterococcus fecalis was 100% sensitive to imipenem, Ciprofloxacin, and Of loxacin but 100% resistant to Amoxicillin, Azithromycin, Gentamycin, Ceftazidime and Cefuroxime .

3. DISCUSSION

Effective management of patients suffering from UTIs commonly relies on the accurate identification of etiological agents and the selection of an appropriate antimicrobial agent. of the 260 urine samples cultured, 98 yielded significant bacterial growths giving an overall prevalence of 37.7%. This prevalence rate was higher when compared to the findings of Shrestha et al. in Nepal 16%, Shaikh et al. 7.8% in the U.S.A, Sumit et al. in North India, 20.73% and 28%, reported by Gautan and Pokhrel in Kitipur, Nepal respectively but lower than the 57.3% reported by Akter et al. in Dhaka, Bangladesh [1,22,23,24,25]. The disparity may have been due to method of collection of sample,

Table 1. Frequencies of the bacterial isolates in the studied group (n=260)

Organisms	Number of isolates	Percentage (%)
E. coli	30	32.6
Klebsiella spp	17	18.5
Citrobacter spp	12	13.0
Staphyococcus aureus	12	13.0
Proteus spp	11	12.0
Pseudomonas aeruginosa	9	9.8
Enterobacter	6	6.5
Enterococcus fecalis	1	1.1
Total	98	100

Table 2. Age and gender distribution of patients

Age(Years)	Gender		Total (%)
	Male (%)	Female (%)	
0-5	14(46.7)	24(35.3)	38 (38.8)
6-10	4(13.3)	8(11.8)	12(12.3)
11-15	6(20.0)	10(14.7)	16(16.3)
16-17	6(20.0)	26(38.3)	32(32.7)
Total	30(30.6)	68(69.4)	98(100)

Table 3. Antimicrobial susceptibility pattern of gram negative organisms

Antibiotics	*E. coli* n= 30	*Klebsiella spp* n=17	*Citrobacter* n= 12	*Proteus* n=11	*Pseudomonas aeuroginosa* n= 9	*Enterobacter* n= 6
OFL	S=16(53.3)	S=12(70.6)	S=3((25)	S=4(36.4)	S=5(55.6)	S=4(66.7)
	R=14(46.7)	R=5(29.4)	R=9(75)	R=7(63.6)	R=4(44.4)	R=2(33.3)
AMOXY	S=0(0)	S=2(11.8) R=15(88.2)	S=2(16.7)	S=1(9)	S=0(0)	S=2(33.3)
	R=30(100)		R=10(83.3)	R=10(90)	R=9(100)	R=4(66.7)
CIPRO	S=18(60)	S=11(64.7)	S=7(58.3)	S=4(36.4)	S=8(88.9)	S=1(16.7)
	R=12(40)	R=6(36.4)	R=5(41.7)	R=7(63.6)	R=1(11.1)	R=5(83.3)
NIT	S=26(86.7)	S=12(70.6)	S=9(75)	S=7(63.3)	S=7(77.8)	S=3(50)
	R=4(13.3)	R=5(29.4)	R=3(25)	R=4(36.6)	R=2(22.2)	R=3(50)
GENTA	S=12(40)	S=10(58.8)	S=5(41.7)	S=3(27.2)	S=5(55.6)	S=2(33.3)
	R=18(60)	R=7(42.2)	R=7(58.3)	R=8(72.7)	R=4(44.4)	R=4(66.7)
IMIPE	S=30(100)	S=17(100)	S=10(83.3)	S=11(100)	S=9(100)	S=6(100)
	R=0(0)	R=0(0)	R=2(16.7)	R=0(0)	R=0(0)	R=0(0)
CEFTAZ	S=14(46.7)	S=6(35.3)	S=3(25)	S=6(54.5)	S=2(22.2)	S=2(33.3)
	R=16(53.3)	R=11(64.7)	R=9(75)	R=5(45.5)	R=7(77.8)	R=4(66.7)
CEFURO	S=8(26.7)	S=5(29.4)	S=3(25)	S=0(0)	S=3(33.3)	S=6(100)
	R=22(73.3)	R=12(70.6)	R=9(75)	R=11(100)	R=6(66.7)	R=0(0)
AUG	S=1(33.3)	S=1(5.9)	S=0(0)	S=1(9)	S=1(11.1)	S=10(90)
	R=29(96.7)	R=16(94.1)	R=12(100)	R=10(90)	R=8(88.9)	R=2(33.3)
CEFTRIX	S=22(73.3)	S=11(64.7)	S=5(41.7)	S=8(72.7)	S=0(0)	S=2(33.3)
	R=8(26.7)	R=6(35.3)	R=7(58.3)	R=3(27.2)	R=9(100)	R=4(66.7)
AZITH	S=16(53.3)	S=3(17.6)	S=3(25)	S=0(0)	S=5(55.6)	-
	R=14(46.7)	R=14(82.4)	R=9(75)	R=11(100)	R=4(44.4)	-
LEVO	S=20(66.7)	S=9(52.9)		S=6(54.5)	S=5(55.6)	S=0(0)
	R=10(33.3)	R=8(47.1)	-	R=5(45.5)	R=4(44.4)	R=6(100)

Table 4. Antimicrobial susceptibility pattern of gram-positive organisms

Antibiotics	*Staphylococcus aureus* No/% n=12	*Enterococcus fecalis* No/% n=1
OFL	S= 3(25)	S =1(100)
	R= 9(75)	R = 0(0)
AMOXY	S = 1 (8.3)	S = 0(0)
	R= 11(91.7)	R= 1(100)
CIPRO	S=10 (83.3)	S =1(100)
	R= 2 (16.7)	R = 0 (0)
NIT	S= 8(66)	S =1(100)
	R= 4(33.3)	R= 0(0)
GENTA	S= 5 (41.7)	S = 0(0)
	R=7 (59.3)	R= 1(100)
IMIPE	S= 12(100)	S =1(100)
	R= 0(0)	R= 0(0)
CEFTAZ	S =3 (25)	S =0(0)
	R=9 (75)	R=1(100)
CEFURO	S= 0(0)	S =0(0)
	R= 12(100)	R= 1(100)
AUG	S = 3 (25)	S =0 (0)
	R= 9 (75)	R= 1(100)
CEFTRIX	S =2 (16.7)	S= 0(0)
	R=10 (83.3)	R 1(100)
AZITH	S= 0(0)	S 0(0)
	R=12(100	1(100)
LEVO	S= 6(50)	0(0)
	R =6(50)	1(100)

hygiene standard, and nutritional status of patients. Many factors have been reported to be the cause of UTI in children; these include the inability to wipe the peri-anal region after a bowel movement, constipation, not completely emptying the bladder, and structural or functional problems.

Gender distribution showed a higher prevalence in females, with a male-female ratio of 1:2.27, and this was found to be statistically significant p <0.05. Several studies in pediatric reported female predominance with a variable ratio ranging from 1:1.3 – 1:6 depending on the difference in sample size and the age group being studied [23]. On the contrary, Gautam et al. reported high prevalence in males (51%) when compared to females (48%) with a ratio of 1:1.06. [26]. High prevalence of urinary tract infections (UTIs) in the female is as a result of many factors such as proximity of the female urethra to the anus, as well as incomplete and inordinate voiding of urine of schoolgirls of which is often associated with constipation and encourages infections of the urinary tract [27].

Age-wise distribution showed that the highest prevalence of UTI was seen in children aged 0-5 years (39.2%). This agrees with the work of Rai et al. that reported the highest prevalence among children less than six years but disagrees with the work of Gautam et al. that reported the highest prevalence among 6-10 years [26,28]. The higher prevalence of UTI among 0-5 years in our work could be because younger children are not well toilet trained with the likelihood of ascending infection from fecal flora [28].

All the significant culture growths were monomicrobial and this aligns with reports in the literature from Nigeria and globally. *Escherichia coli* was the most common isolated organism accounting for 32.6% (n=30) followed by *Klebsiella spp* 18.5% n=17), *Citrobacter* 13% (n=12), *Proteus spp* 12% (n=11), *Pseudomonas aeruginosa* 9.8% (n=9) and the least was *Enterobacter* 6.5% (n=6). This finding aligns with the work by Nair and Rai, Shrestha *et al* and Gautam and Pokhrel who reported the predominance of *E. coli* [29,1,24]. The same pattern was also observed by Gautam et al. and Sumit et al. [26,23]. *Enterobacteriaceae* have several factors responsible for their attachment to the uroepithelium. These Gram-negative aerobic bacteria colonize the urogenital mucosa with adhesin, pili, fimbriae and PI-blood group phenotype receptors [21]. Among the Gram-positive organisms, *Staphylococcus* aureus was the most common 12(13%) followed by *Enterococcus fecalis* 1 (1.1%).

The antimicrobial sensitivity and resistance pattern varies from community to community and from hospital to hospital. Indiscriminate and abuse of antibiotics have led to the emergence of resistant strains [17,21]. The percentage of resistances of different antimicrobial agents varies from 0% to 100% in our study. Higher resistance was noted among Amoxicillin, Augmentin, Cephalosporins, Gentamycin, and Azithromycin classes of drugs, and this limits their usefulness in UTI treatment.

E. coli showed the highest resistance to amoxicillin 100%, Augmentin 96.7%, Cefuroxime 73.3% Gentamicin 60%. This resistance may have been due to inappropriate use of these drugs [17,21]. *Klebsiella* and *E. coli* showed almost the same resistance pattern.

Gram-positive organisms in our study showed high resistance to Azithromycin 100%, Cefuroxime 100%, Amoxicillin 91.7% and Ceftriaxone 83.3%. From the result of this study it could be said that drugs like Amoxicillin, Augmentin and Azithromycin, Ceftazidine have almost lost their relevance in UTI treatment probably due to overuse and/or misuse of antibiotics.

There is a high sensitivity of organisms to Imipenem and Nitrofurantoin. Nitrofurantoin may be used to treat lower UTIs but because of its limited tissue penetration, it is not suitable for the treatment of kidney infection [1]. It is not also recommended in infants younger than 6 weeks [1].

4. CONCLUSION

The overall prevalence of UTI in our study was 37.7%. Females were more susceptible to UTI with 26.2% prevalence, as against 11.5% in males.

Escherichia coli were the most predominant aetiological agents in this study causing 32.6% of infection. Other aetiological agents include *Klebsiella pneumoniae, Citrobacter spp.*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter spp*, *Staphylococcus aureus*, and *Streptococcus spp.*

Imipenem was found to be the most sensitive antibiotics followed by Nitrofurantoin and Ciprofloxacin. There is a need to regularly monitor the antimicrobial susceptibility profile of these etiological agents to keep track of the effectiveness of certain therapeutic agents.

CONSENT AND ETHICAL APPROVAL

The study was examined and approved by the Ethics Committee of UNTH. The work was performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

Informed and written consent was sought and obtained through writing from the patient's parents/ guardians.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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