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Prevalence and Antibiotic Resistance Patterns of Gram-Negative Uropathogens among Paediatric Patients in Nigeria

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Abstract

Objective: This study aimed to detect and compare the frequency and antibiotics resistant pattern of Gram-negative uropathogens implicated in urinary tract infections (UTIs) in paediatric patients attending some hospitals in Nigeria and to proffer recommendations for its management. **Methods:** Based on standard procedures, midstream urine samples were collected. Urinalysis was done as a preliminary diagnosis of UTI using Combi-9 test strip. Isolation of uropathogen was done and antibiotic sensitivity test was carried out using Kirby-Bauer technique. **Results:** Out of 489 samples collected, 130 (26.4%) was positive for UTI. The prevalence rate of UTI in the investigated areas such as Nsukka, Otukpo, Gboko and Kastina Ala was 31.8%, 17.5%, 34.3% and 17.1%, respectively. The prevalence of UTI was higher in males 81 (30.9%) than in females 49 (21.6%), but there was no statistically significant association between gender and UTI (p = 0.636). The prevalence of UTI was greater among the age of 2 - 5 years (28.2%) and decreased with the increase in age, although there was no significant association between UTI and the age groups (p = 0.870). Generally, *Klebsiella pneumonae* (88.8%) was the most dominant bacterium (it was even more in males), followed by *E. coli* (40.6%), which was more in females, then *Pseudomonas* spp. (45.0%) and *Proteus mirabilis* (13.8%). The *in-vitro* antibiotic susceptibility testing shows that the isolate was highly resistant to Augmentin, Cotrimoxazoel, Amoxicilin and Tetracycline, while some of the isolate shows intermediate resistant to Nitrofurantoin and Nalixidic acid. Ofloxacine and Gentamicin were the most effective antibiotics against the isolates from all the study areas. The isolates

had a varied range of MICs and MBCs. **Conclusion:** *K. pneumonia* predominated all isolates. The resistant patterns of the isolates to some of the antibiotics show that the first line of antibiotics for treatment of UTI in children in these areas is Ofloxacine and Gentamicin.

Keywords

Urinary Tract Infection, Bacteria, Gram Negatives, Enterobacteriaciae, Antibiotics Resistance/Susceptibility

1. Introduction

Urinary tract infection (UTI) is regarded as one of the most serious infections caused by bacteria especially in children population. For some years now, the significance of UTI in children has been progressively acknowledged and diagnosed [1]. Additionally, when antimicrobial therapy is given to a child having UTI appropriately and promptly, a rapid recovery would be achieved and some acute and/or persistent consequences, which include renal scarring, renal function, as well as hypertension, should be averted [2].

Apart from *Staphylococcus aureus* and *Enterococcus*, infections of bacteria are also caused by members of the Enterobacteriaciae which include *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* spp. and *Pseudomonas aeruginosa* [3] [4] [5]. Presently, it is worrisome of the pattern with which these bacterial pathogens resist commonly used antibiotics or antimicrobial agents [1].

Diagnosis of UTI relies on both urinalysis and urine culture; the diagnosis of UTI in children is normally complicated and may be missed. This is because the clinical presentation of UTI may be insignificant and sometimes with nonspecific clinical signs which may include abdominal pain as well as fever that may as well be seen in various acute self-limiting viral illnesses during childhood [2] [6] [7]. For the UTI diagnosis to be confirmed in a laboratory prior to treatment, urine culture as well as testing for antibiotics sensitivity is usually done. But it challenges to collect uncontaminated urine samples from children and infants, who are not toilet trained [2] [8]. In treatment of UTI in children, there are no well-established antibiotics of choice since the sensitivity of the pathogens differs in several locations [9] [10].

In Nigeria, an extensive investigation has not been done the resistance pattern of uropathogens, especially in children, although, there are some studies in some location/healthcare centres in few states [7] [11] [12]. It is in this view that this study was designed to extend the frontiers of available medical information in the areas of UTIs. It is aimed at investigating the prevalence of these Gram-negative bacteria responsible for UTI among children in the areas under study and exploring sensitivity patterns of identified microorganisms to certain antibiotics used in the treatment of UTI.

2. Materials and Methods

The study is a hospital based cross-sectional study conducted between December and April. Ethical approval was obtained from the Chief Medical Director and Ethical Committee of these hospitals: Chidubem Children Specialist Hospital Nsukka, Bishop Shanahan Hospital, and Ochil Hospital, Nsukka, Enugu State; General hospital in Katsina Ala, General hospital in Alaide, St. Vincent hospital, and Comprehensive Health Centre in Benue State. The concept form was given to each patient before samples were collected. Inclusion criteria: patients who are within the age range of 2 - 12 years only. Exclusion criteria: children below one year, above 12 years, and patients who have been on antibiotics for at least three days before the day of sample collection. Midstream urine samples were collected randomly from in and outpatients using sterile universal bottles. Samples were delivered to the laboratory within an hour.

2.1. Isolation and Identification of Urine Samples

About 0.001 ml of samples where inoculated into Nutrient agar (Oxoid, United Kingdom) and MacConkey agar (Oxoid, United Kingdom) using micropipette. The media were prepared according to the manufacturer's instructions. After inoculating sample on culture plates, urine microscopy was also done. At 2000 g for 5 min, each urine samples (5 - 10 ml) were centrifuged (using Teco diagnostic centrifuge), followed by microscopic examination (×40 objective) of the wet preparation of the sediment. The presence of pyuria (more than 5 white blood cells per high power field (HPF)) or any bacteria per HPF was noted as significant and suggestive of UTI [2]. Under aerobic condition at 37˚C for 24 hours, the plates were incubated and colony forming units (CFU) was determined and those that had $\geq 10^5$ CFU/mL were considered to be significant indication of UTI and were marked for further investigation. The isolates were confirmed with Chromogenic media (Uriselect^{tm} 4 Agar Bio-Rad Laboratory).

Using sterile wire loop, colonies were streaked on prepared molten CHROM agar plates and were incubated aerobically for 24 hours for colour change. Identification of each organism was done following the manufacturer's colour guide; further identification was carried out using biochemical tests following standard techniques [13].

2.2. Antibiotic Susceptibility Test

The antibiotics susceptibility test was performed using the Kirby Bauer disk diffusion technique [14] with commercially available disks (ABTEK laboratories). A 0.1 mL of 0.5 MacFarland standardized isolates (approximately 10^7 CFU/mL) was used and the plates were incubated at 37˚C for 16 - 18 h. And according to Clinical Laboratory Scientific Institute (CLSI) approved standard guidelines, the inhibition zones diameter of the antibiotics were measured, recorded and interpreted. The antibiotics tested against Gram-negative bacterial isolate were Augmentine Ofloxacine Gentamicin, Nalixidic Acid, Nitrofurantoin, Cotrimoxazole, Amoxicilin and Tetracycline.

2.3. Minimum Inhibitory Concentration (MIC)

Following the initial antimicrobial screening tests, the minimum inhibitory concentrations of each antibiotic were determined by using broth tube microdilution method as described by Andrew [15], in accordance with CLSI approved standard for bacteria. The antibiotics were dissolved in their different appropriate diluent. Ofloxaxine 500 mg was dissolved in 500 mls of sterile distil water, gentamycin was dissolved in distil sterile water, while nitrofurantoin 100 mg was dissolved in 100 mL of Dimethylsulphuramide (DMF). 744 µl of sterile Mueller Hinton broth was added to 256 µl of antibiotics *i.e.* ofloxacine, gentamycin and nitrofurantoin given a total of 1000 µl. another 1000 µl of sterile Mueller Hinton broth is added to the tube containing the antibiotics and the broth to reduce the concentration to 128, then 1000 µl of is pipette into another sterile test tube given a concentration of 128. Another 1000 µl of sterile Mueller Hinton broth is added to the tube containing 128 concentrations to reduce the concentration to 64 and then serially transferring 1000 µl from it to the next tube and so on for 16, 8, 4, 2, 1, 0.5 and 0.25 µg/ml is achieved for the different antibiotics, about 1000 µl was removed from the last tube and discarded. 700 µl of the serially diluted different concentrations is pipette into the sterile eppendorf tubes then 10 µl of the standardized test organism were dispensed into the eppendorf tubes containing the different concentrations. The negative control tubes were different concentrations of antibiotics in MHB without the test isolates, while the positive control tubes contained 1000 µl broth medium and each of the test organisms. All the test tubes were incubated for 18 to 24 hours at 37˚C. The MIC was determined visually by inspecting the tubes for turbidity post-incubation (matching the test tubes with test organisms with the corresponding negative control tubes). The MIC was reported as the lowest concentration of the test antibiotics which resulted in 100% inhibition of the test organism.

2.4. Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the antibiotics was determined by further sub-culturing from the tubes which showed no visible growth in the MIC assay onto freshly prepared Mueller Hinton agar (MHA) plates. The culture plate containing bacteria were incubated at 37˚C for 24 hours. The MBC was therefore taken as the lowest concentration that did not show any visible growth on the sub-cultured MHA plate [15].

2.5. Determination of Multiple Antibiotic Resistance Index (MARI)

Multiple Antibiotic Resistance Index (MARI), has been shown to be a cost effective and valid method of bacteria source tracking. Multiple antibiotic resistance indexes are calculated as the ratio of number of antibiotics to which an organism is resistant to total number of antibiotics to which the organism has been exposed [16]. The MAR indices of the isolates were calculated and noted.

2.6. Data Analysis

Results were analysed using SPSS software version 25.0 for Windows (SPSS Inc. 2017 Chicago, Illinois, USA). Categorical variables were tested for association using Pearson Chi-square and Fisher exact test as appropriate. Significant level was set at *P* value of 0.05.

3. Results

3.1. Prevalence of Uropathogen in the Study Areas

Of the 489 urine samples examined, the overall prevalence of urinary tract infection was 26.4%. The prevalence rate of UTI in the investigated areas such as Nsukka, Otukpo, Gboko and Kastina Ala was 31.8%, 17.5%, 34.3% and 17.1%, respectively (Table 1). Out of 130 positive samples, the prevalence of UTI was

Table 1. Prevalence of UTI among children attending some hospitals in the study areas based on sex and age.

Location	Parameter	Gender		Total	Age (Year)			Total
		Male	Female		2 - 5	6 - 9	10 - 12	
Nsukka	NE	121	102	223	127	62	34	223
	NI	43	28	71	40	20	11	71
	P (%)	35.5	27.5	31.8	31.5	32.3	32.4	31.8
	X^2	0.45			51.75			
	p-value	0.49			0.00			
Otukpo	NE	64	62	126	65	27	34	126
	NI	15	7	22	18	1	3	22
	P (%)	24	11.2	17.6	27.7	3.7	8.8	17.5
	X^2	2.28			109.36			
	p-value	0.10			0.00			
Gboko	NE	43	27	70	34	21	15	70
	NI	17	7	24	10	10	4	24
	P (%)	39.5	25.9	34.3	29.4	47.6	26.7	34.3
	X^2	0.81			33.46			
	p-value	0.37			0.00			
Kastina Ala	NE	34	36	70	40	20	10	70
	NI	6	7	13	7	4	2	13
	P (%)	17.6	19.4	18.6	17.5	20	20.0.	18.6
	X^2	0.05			50.01			
	p-value	0.81			0.00			
Overall	NE	262	227	489	266	130	93	489
	NI	81	49	130	75	35	20	130
	P (%)	30.9	21.6	26.6	28.2	26.9	21.5	26.6
	X^2	0.82			0.28			
	p-value	0.36			0.87			

NE: Number examined, NI: Number infected, P: Prevalence, Statistically significant at $p < 0.05$.

higher in males 81 (30.9%) than in females 49 (21.6%), but there was no statistically significant association between gender and UTI ($p = 0.636$) (Table 1). The age categorizations of the study subjects were 2 - 5, 6 - 9 and 10 - 12. This study revealed that the prevalence of UTI was greater among the age 2 - 5 (28.2%), followed by the age group 6 - 9 (26.9%), while the age group 10 - 12 had the least prevalence of 21.5% (Table 1); although there was no statistically significant association between UTI and the age groups ($p = 0.870$).

The result of dipstick urinalysis showed that out of 489 sample examined, 36 (7.4%) was positive for nitrite; 33 (6.8%) for bilirubin, 8 (1.6%) for blood, 10 (2.0%) for urobilinogen, 111 (22.7%) for protein, 31 (6.4%) for ketone, 86 (17.6%) for ascorbic acid and 22 (4.5%) for glucose. The pH categorisation shows that 292 (59.8%) had acidic pH urine followed by 142 (29.1) for neutral and 53 (10.9%) alkaline pH (Table 2).

3.2. Microorganisms Isolated from Urine Culture

The prevalence of uropathogens among children attending hospitals in all the study areas according to urine samples culture, showed that four Gram-negative organisms were isolated and they are *Klebsiella pneumonae*, *E. coli*, *Pseudomonas* spp. and *Proteus mirabilis* (Figure 1(a)). *Klebsiella pneumonae*, was the most predominant organism (88.8%) and it was more isolated from male children, followed by *E. coli.* It was observed that *E. coli* was the most predominant organism isolated from the female children (40.6%), and also *Pseudomonas* spp was seen more in male children (45.0%) and *Proteus mirabilis* was the least (13.8%) (Figure 1(a)). The bacteria with highest prevalence in Nsukka, Otukpo, Gboko, and Kastina Ala were *Klebsiella pneumonae* (46.4%) (Figure 1(b)), *Klebsiella* spp (58.3%) (Figure 1(c)), *E. coli* (38.5%) (Figure 1(d)), and *Klebsiella* spp (42.0%) (Figure 1(e)), respectively.

Table 2. Urinalysis of samples collected from children attending some hospitals.

Urinalysis		Prevalence (%)				
		Nsukka	Otukpo	Gboko	Kastina Ala	Overall
Blood		1 (1.7%)	4 (3.2%)	3 (4.3%)	3 (4.3%)	8 (1.6)
Urobilinogen		6 (3.3%)	3 (2.4%)	1 (1.4%)	1 (1.4%)	10 (2.0)
Bilirubin		14 (8.3%)	8 (6.4%)	7 (10%)	7 (10%)	33 (6.8)
Protein		59 (35%)	20 (16%)	22 (31.4%)	22 (31.4%)	111 (22.7)
Nitrite		14 (8.3%)	14 (11.2%)	1 (1.4%)	1 (1.4%)	36 (7.4)
Ketone		23 (13.3%)	1 (0.8%)	3 (4.3%)	3 (4.3%)	31 (6.4)
Ascorbic acid		42 (25%)	17 (13.6%)	20 (28.6%)	20 (28.6%)	86 (17.6)
Glucose		1 (1.7%)	6 (4.8%)	11 (15.7%)	11 (15.7%)	22 (4.5)
pH	Acidic	138 (81.7%)	84 (67.2%)	25 (35.7%)	25 (35.7%)	292 (59.8)
	Neutral	62 (36.7%)	34 (27.2%)	22 (31.4%)	22 (31.4%)	142 (29.1)
	Alkaline	23 (13.3%)	7 (5.6%)	22 (31.4%)	22 (31.4%)	53 (10.9)
		N = 223	N = 126	N = 70	N = 70	N = 489

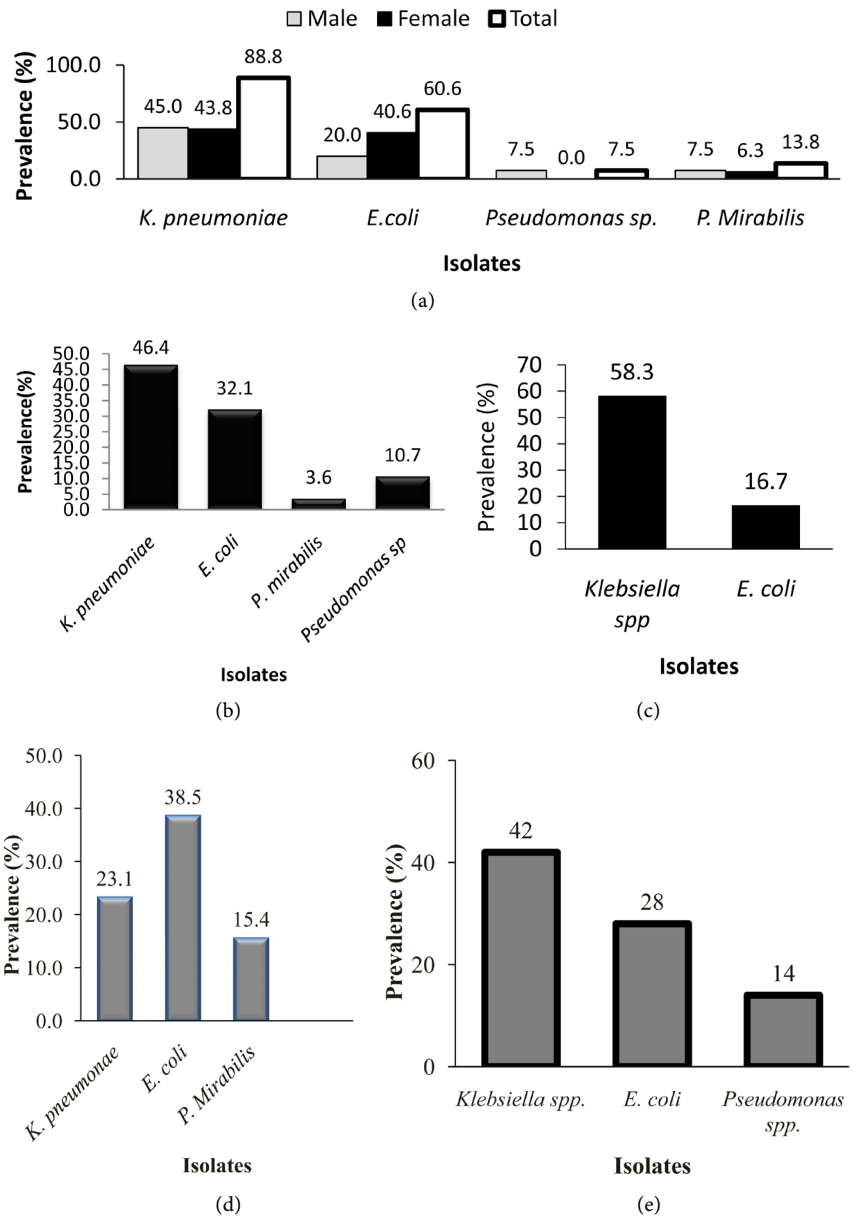


Figure 1. (a) Overall frequency of the bacterial isolates from children with UTI; (b) in Nsukka; (c) Otukpo; (d) in Gboko; (e) in Kastina Ala.

Evaluation of antibiotic susceptibility patterns of isolates to antibiotics used in the treatment of UTI in children presenting at some hospitals in the study areas.

The *in-vitro* antibiotic susceptibility tests were made on a total of 52 randomly selected isolates, which includes 16 from Nsukka, 12 from Otukpo, 16 from Gboko and 8 from Kastina Ala. This study showed that most of the isolate were highly resistant to Augmentin, Cotrimoxazoel, Amoxicilin and Tetracycline, while some of the isolate shows intermediate resistant to Nitrofurantoin and Nalixidic acid. Ofloxacine and Gentamicin were the most effective antibiotics against the isolates from all the study areas (Supplementary materials: Tables 1-4). *K. pneumoniae* isolates from Nsukka (56.3%), Otukpo (55.6%) and K. Ala

Table 3. Overall percentage antibiotic susceptibility pattern of Gram-negative bacteria isolates.

Loaction	Isolates	Susceptible (%)	Intermediate (%)	Resistant (%)
Nsukka	*E. coli*	9 (28.1)	6 (18.8)	17 (53.1)
	K. pneumonia	16 (25.0)	12 (18.8)	36 (56.3)
	P. mirabilis	2 (25.0)	2 (25.0)	4 (50.0)
	Pseudomonas spp.	4 (16.7)	3 (12.5)	17 (70.8)
Otukpo	*E. coli*	9 (37.5)	1 (4.2)	14 (58.3)
	K. pneumonia	26 (36.1)	6 (8.3)	40 (55.6)
Gboko	*E. coli*	19 (39.6)	3 (6.3)	26 (54.2)
	K. pneumonia	10 (31.3)	6 (18.8)	16 (50.0)
	Proteus mirabilis	11 (34.4)	2 (6.3)	19 (59.4)
K. Ala	*E. coli*	9 (37.5)	3 (12.5)	12 (50.0)
	K. pneumonia	9 (28.1)	6 (18.8)	17 (53.1)
	Pseudomonas spp.	3 (37.5)	0 (0)	5 (62.5)

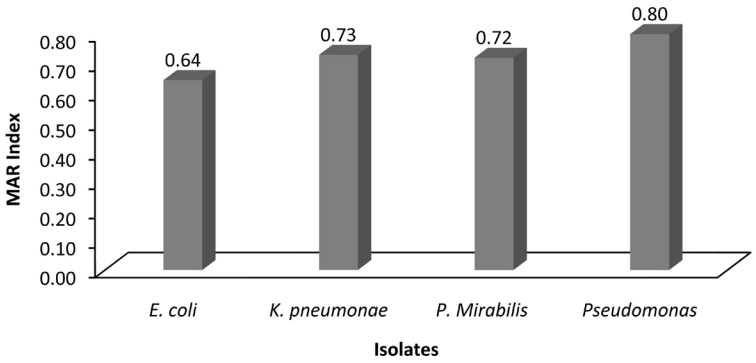
Table 4. Summary of antibiotic susceptibility pattern of all Gram-negative bacteria isolates to the antibiotics.

Isolate	R/S (%)	AUG	OFL	GEN	NAL	NIT	COT	AMO	TET
E. coli	S	0.0	84.6	84.6	23.1	100.0	0.0	0.0	16.7
	R	100.0	15.4	15.4	76.9	0.0	100.0	100.0	83.3
K. pneumonia	S	0.0	81.5	59.3	3.7	63.0	11.1	0.0	22.2
	R	100.0	18.5	40.7	96.3	37.0	88.9	100.0	77.8
P. mirabilis	S	0.0	80.0	100.0	20.0	40.0	0.0	0.0	20.0
	R	100.0	20.0	0.0	80.0	60.0	100.0	100.0	80.0
Pseudomonas spp.	S	0.0	75.0	25.0	25.0	50.0	0.0	0.0	0.0
	R	100.0	25.0	75.0	75.0	50.0	100.0	100.0	100.0

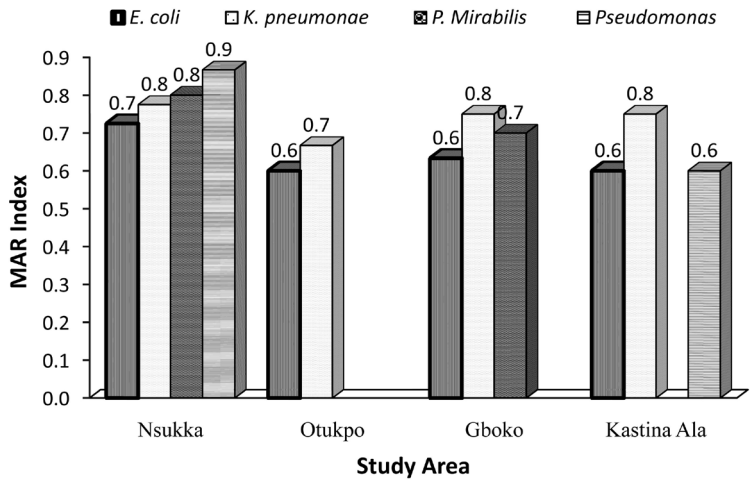
KEY: R = Resistant (+Intermediate), S = Sensitive. AUG = Augmentin, OFL = Ofloxacin, GEN = Gentamicin, NAL = Nalixidic Acid, NIT = Nitrofurantoin, COT = Cotrimoxazole, AMO = Amoxicilin, TET, Tetracycline.

(53.1%) were the most resistant to the test antibiotics, while *E. coli* isolates from Gboko had highest percentage of resistance (Table 3). More than 50% of each of the bacteria isolates were resistant to NAL, COT and TET (Table 4).

Overall average MAR index of all the isolates from the different region revealed that *Pseudomonas*sp had the highest MARI of 0.80, followed by *K. pneumoniae* (0.73), and *P. mirabilis* (0.72) and *E. coli* (0.64) (Figure 2(a)). The average MAR index of isolates from the different study areas shows that *Pseudomonas*sp isolates from Nsukka had the highest MARI at 0.9, followed by *K. pneumoniae* (all locations) and *P. mirabilis* (Nsukka) (0.8). *E. coli* had the least MAR index of 0.6 (Figure 2(a)).



(a)



(b)

Figure 2. (a) Overall average MAR index of isolates; (b) Average MAR index of isolates from different study areas.

3.3. MIC and MBC of the Antibiotics

The MICs of nitrofurantoin against *Pseudomonas* sp, *K. pneumoniae*, *P. mirabilis*, and *E. coli* isolates from all the study areas ranged from 8.0 - 128.0, 16.0 - 128.0, 16.0 - 64.0, and 8.0 - 64.0 µg/ml, respectively, while the MBCs were 64.0- > 128.0, 32.0- > 128.0, 128.0- > 128.0, and 32.0- > 128.0 µg/ml, respectively (Tables 5-8). The MICs of ofloxacine against the sameisolates ranged from 4.0 - 16.0, 4.0 - 32.0, 8.0 - 16.0, and 0.5 - 16.0 µg/ml, respectively, while the MBCs were 8.0 - 128.0, 16.0 - 128.0, 16.0 - 64.0, and 8.0 - 32.0 µg/ml, respectively (Tables 5-8). The MICs of gentamicin against the sameisolates ranged from 4.0 - 16.0, 8.0 - 64.0, 8.0 - 64.0, and 8.0 - 32.0 µg/ml, respectively, while the MBCs were 32.0- > 128.0, 32.0- > 128.0, 32.0 - 128.0, and 16.0 - 64.0 µg/ml, respectively (Tables 5-8).

4. Discussion

UTI is one of the infections frequently encountered in hospitals and diagnostic laboratories [17]. Therefore, performing area-specific monitoring studies is essential which may aid in choosing the correct empirical treatment for the

Table 5. The MIC and MBC of antibiotics against isolates from Nsukka.

Isolates	Nitrofurantoin (µg/ml)		Ofloxacine (µg/ml)		Gentamicin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Pseudomonas sp 4	32.0	>128.0	8.0	32.0	32.0	64.0
K. pneumoniae 39	32.0	>128.0	16.0	64.0	64.0	128.0
P. mirabilis 38	16.0	>128.0	8.0	16.0	8.0	32.0
E. coli 29	16.0	64.0	2.0	8.0	8.0	16.0
K. pneumoniae 92	64.0	>128.0	16.0	64.0	32.0	128.0
K. pneumoniae 47	16.0	32.0	8.0	16.0	16.0	32.0
Pseudomonas sp 51	128.0	>128.0	32.0	128.0	64.0	>128.0
E. coli 40	64	128.0	0.5	8.0	8.0	32.0
K. pneumoniae 55	32.0	128.0	64.0	128.0	64.0	>128.0
E. coli 80	32.0	>128.0	2.0	32.0	16.0	64.0

>128.0 = No MBC; MIC-Minimum inhibitory concentration; MBC-Minimum bactericidal concentration.

Table 6. The MIC and MBC of antibiotics against isolates from Otukpo.

Isolates	Nitrofurantoin (µg/ml)		Ofloxacine (µg/ml)		Gentamicin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli 10	32.0	>128.0	2.0	16.0	8.0	64.0
K. pneumoniae 77	>128.0	>128.0	16.0	64.0	64.0	>128.0
K. pneumoniae 49	64.0	>128.0	4.0	16.0	8.0	32.0
E. coli 60	64.0	64.0	4.0	8.0	16.0	32.0
K. pneumoniae 5	64.0	>128.0	32.0	128.0	32.0	>128.0
E. coli 34	16.0	32.0	16.0	32.0	16.0	32.0
K. pneumoniae 28	128.0	>128.0	32.0	64.0	64.0	>128.0
E. coli 20	64	128.0	4.0	16.0	16.0	64.0
E. coli 11	8.0	64.0	8.0	32.0	32.0	64.0
K. pneumoniae 98	64.0	>128.0	32.0	64.0	64.0	128.0

>128.0 = No MBC; MIC-Minimum inhibitory concentration; MBC-Minimum bactericidal concentration.

Table 7. The MIC and MBC of antibiotics against isolates from Gboko.

Isolates	Nitrofurantoin (µg/ml)		Ofloxacine (µg/ml)		Gentamicin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
K. pneumoniae 55	32.0	>128.0	8.0	16.0	8.0	32.0
K. pneumoniae 30	128.0	>128.0	32.0	64.0	64.0	>128.0
P. mirabilis 7	64.0	>128.0	16.0	64.0	64.0	128.0
E. coli 10	16.0	64.0	8.0	8.0	16.0	32.0
K. pneumonia 17	128.0	>128.0	32.0	128.0	32.0	>128.0
E. coli 20	32.0	64.0	16.0	32.0	32.0	64.0
P. mirabilis 41	32.0	128.0	8.0	16.0	16.0	64.0
E. coli 39	32.0	128.0	8.0	16.0	16.0	64.0
E. coli 60	64.0	128.0	16.0	32.0	32.0	64.0
P. mirabilis 62	64.0	>128.0	16.0	64.0	64.0	128.0

>128.0 = No MBC; MIC-Minimum inhibitory concentration; MBC-Minimum bactericidal concentration.

Table 8. The MIC and MBC of antibiotics against isolates from Kastina Ala.

Isolates	Nitrofurantoin (µg/ml)		Ofloxacine (µg/ml)		Gentamicin (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
K. pneumonia 15	64.0	>128.0	16.0	64.0	32.0	128.0
Pseudomonas sp 2	64.0	128.0	4.0	8.0	16.0	32.0
Pseudomonas sp 10	64.0	128.0	16.0	32.0	32.0	64.0
E. coli 45	32.0	64.0	8.0	32.0	32.0	64.0
E. coli 70	16.0	32.0	4.0	16.0	16.0	32.0
Pseudomonas sp 65	8.0	64.0	8.0	32.0	32.0	64.0
E. coli 30	32.0	64.0	8.0	8.0	16.0	32.0
K. pneumoniae 25	64.0	>128.0	32.0	128.0	32.0	64.0
K. pneumoniae 54	64.0	>128.0	32.0	64.0	64.0	128.0

>128.0 = No MBC; MIC-Minimum inhibitory concentration; MBC-Minimum bactericidal concentration.

infection/disease. In this study, the overall prevalence of UTI among children attending hospitals in the areas was 26.4%. Highest prevalence rate of UTI was observed among children attending hospitals in Gboko (34.3%), followed by Nsukka (31.8%), Otukpo (17.5%) and Kastina Ala (17.1%). The observed UTI prevalence rate in Gboko and Nsukka including overall prevalence, are higher than prevalence rate of UTI (24.7%) reported byDada and Aruwa [18] for children (5 - 11 years) in Ondo State, Nigeria. In another study in Ebonyi State, Moses *et al.* reported a higher prevalence rate of 48% among school children (4 - 12 years) in a rural area [7].

Consequently, on the basis of laboratory tests of urine using urine dipstick, it was found that 36 (7.4%), 8 (1.6%), 10 (2%) and 33 (6.8%) were positive for nitrite, blood, urobilinogen and bilirubin respectively, which may be an indication of UTI, haematuria, or viral infection either hepatitis or jaundice [19].

We observed that the overall prevalence of bacteriuria was higher in male (30.9%) than in female (21.6%) children, even in some study areas like Nsukka, Otukpo and Gboko, contrary to what has been reported in some literature. But in Kastina Ala, female children had higher rate of UTI than their male counterpart. Similarly, higher prevalence rate of UTI in male than female infants have been reported in Brazil (male 3.7%, female 2%) [20], Korea (Male 74%, female 26%) [21] and Nepal (male 53%, female 47%) [8]. The immune system that has not fully developed and exposures to soil and faecal pathogen may be some of the predisposing factors to UTI in infants/children. UTI rate at neonate and infant is usually higher in males than in females, and in this study, more samples were got from infants, which may have resulted in higher rate in males. In contrary, higher UTI prevalence rates in female than in male children have been reported in Ondo State [22] and Osun State, Nigeria [12] and even in other countries [9] [23]. The prevalence rate of UTI decreased with increase in age of the children, and in our study the infants (2 - 5 years) had the highest prevalence

rate which is similar to other reports [8] [24]. Our study however demonstrated significant association between age differences in the occurrence of UTI among children under the age of 12 years in all the regions under study ($p = 0.000001$).

In this study, the most predominant bacterium recovered from the urine of the children was *Klebsiella pneumonia*, followed by *E. coli*, *Pseudomonas spp.* and *P. mirabilis* (**Figure 1(a)**). *K. pneumonia*, *Pseudomonas spp.* and *P. mirabilis* were more in male children while *E. coli* was more in female children. The pattern and frequency of occurrence of the bacterial isolates found in this study are similar to those reported previously [25]. On the contrary, many studies have reported that the most predominant and frequently isolated bacteria are *Staphylococcus aureus* [7] [26], and *E. coli* [8] [9] [18] [23] [27] [28].

The isolates tested were 100% resistant to augmentin and amoxicillin but susceptible to ofloxacin followed to some extent by gentamycin and nitrofurantoin (which makes them the first line antibiotics for treatment of UTI in children). In addition, 76.9%, 100% and 83.3% of *E. coli* isolates were resistant to NAL, COT and TET, respectively. *K. pneumonia* (96.3%, 88.9% and 77.8%, respectively), *P. mirabilis* (80.0%, 100% and 80%, respectively), and *Pseudomonas* spp. (75%, 100% and 100%, respectively) isolates were resistant to the same drugs. Similar to our finding, there are reports on the effectiveness of gentamycin and ofloxacin against *E. coli* [6] [29] and its resistant to TET, COT, NAL, AMO, NIT [6]. A study in Nigeria reported susceptibility of similar isolates to OFL NIT, and their resistance to TET and COT [30]. Merga Duffa *et al.* and Nazme *et al.* have reported the resistance of some Gram-negative isolates to NAL [5] and COT [27] [28] and the susceptibility of *P. mirabilis* to the same drug [5], similar to our findings. On the contrary, the resistance of *E. coli* to OFL [8] and *K. pneumonia* to NIT but susceptible to AMO [31] have been reported, as well as the suceptiblility of *E. coli*, *K. pneumonia* and *P. mirabilis* to NAL [32]. Pouladfar *et al.* investigation showed that *K. pneumonia* was susceptible to TET, and similar to our findings resistant to AMO, COT, NIT, although, *Pseudomonas* sp. was resistant to NAL and GEN [6]. The sensitivity to NIT in our study is not in tandem with other reports which showed that *P. mirabilis* and *P. aeruginosa* were resistant to NIT [26].

5. Conclusions

The most predominant organism in this study is *K. pneumonia*, followed by *E. coli*, *Pseudomonas spp. and P. mirabilis.* In addition, *K. pneumonia* and *Pseudomonas* spp predominated more in male than in female children. The resistant patterns of the isolates to some of the antibiotics show that the first line of antibiotics for treatment of UTI in children in these areas should be Ofloxacine and Gentamicin.

The observed antibiotic resistant patterns may be due to long term use of some of these drugs over the years. Also, prescriptions of antibiotics without laboratory guidance as well as over the counter sales of antibiotics without prescription are rife in the Nigerian setting.

Availability of Data and Materials

Data are all contained within the paper. The datasets from the analyses are available from the corresponding author on a reasonable request.

Consent for Publication

Not applicable.

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Conflicts of Interest

The authors declare that they have no competing interests.

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