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A STUDY ON SIGNIFICANT BACTERIURIA AMONG CHILDREN ATTENDING THE OUT-PATIENT CLINIC OF A UNIVERSITY TEACHING HOSPITAL, NIGERIA

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Abstract- Significant bacteriuria refers to the laboratory findings of >10^5 colony-forming units (CFU) of bacteria per mL of urine which is diagnostic of urinary tract infection. This study was done to determine significant bacteriuria among paediatric patients attending Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria. Midstream urine was collected from 300 paediatric patients between the ages of 1 to 16 yrs. at Nnamdi Azikiwe University Teaching Hospital Nnewi. Culture plates with bacteria counts greater than or equal to 1 x 10^5cfu ml^{-1} were taken as positive which indicates urinary tract infection (UTI). The bacteria isolates were identified based on colony morphological characteristics as well as biochemical tests using API staph and Rapid ID 32E for Gram positive and Gram negative isolates respectively. Antimicrobial susceptibility test was carried out according to clinical and laboratory standards institute (CLSI). The result of this study showed 8% prevalence of significant bacteriuria among children. Twenty-four children had significant bacteriuria; 16(66.7%) were from female participants while eight (33.3%) were from male participants. *Staphylococcus aureus* 7(29.17%) was the most predominant organism, followed by *Escherichia coli* 6 (25%), *Klebsiella pneumoniae* 5(20.83%), *Staphylococcus xylosus* 3(12.5%), *Pseudomonas* 1(4.17%), *Klebsiella oxytoca* 1(4.17%) and *Staphylococcus chromogenes* 1(4.17%). Urine microscopy showed that 24 specimens had significant bacteriuria. All the isolates were susceptible to imipenem. Significant bacteriuria in children has shown an increasingly prevalence. With avert of antimicrobial resistance, there are bound to be serious public health problems if proper control measures are not implemented.

Keywords- Significant Bacteriuria, Paediatric and Antimicrobial resistance

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Introduction

Urinary tract infection in the pediatric population (less than or equal to18 yrs.) is well recognized as the cause of acute morbidity and chronic medical conditions such as renal insufficiency at adulthood [1]. Urinary tract infections may involve just the lower tract or both the lower and upper tracts. Cystitis refers to infection of the bladder with signs and symptoms including dysuria, urgency and frequency. Pyelonephritis is the term used to describe UTI, often with flank pain, tenderness with accompanying dysuria, urgency and frequency. Cystitis and pyelonephritis often present as acute diseases however, recurrent or chronic infections occur frequently [2].

It is generally accepted that 10^5 or more CFU/mL of urine is significant bacteriuria, though the present maybe symptomatic or asymptomatic [3]. The prevalence of bacteriuria is 1-2% in school aged girls. The prevalence of bacteriuria increases with age, and the sex ratio of infections become nearly equal [4]. Upper urinary tract infections routinely occur in patients with indwelling catheters even with optimal care and closed drainage systems: 50% after 4-5 days, 75% after 7-9 days and 100% after 2 weeks. Studies have shown that sexual activity and spermicide use increases the risk for UTIs in young girls [5].

In complicated upper tract infections most especially in an anatomic settings or chronic catherization, the spectrum of infecting bacteria is larger than in uncomplicated cases [6]. *Escherichia coli* are the most prevalent causative agent although other gram negative rods of several species (eg; *Klebsiella, Proteus, Enterobacter* and *Pseudomonads*), *Enterococci* and *Staphylococci* has show a relatively high prevalence. In several cases, two or more species are present and the bacteria are often resistant to antimicrobials administered in association with prior therapy [7].

The presence of bacteriuria is confirmed by quantitative culture of the urine. One frequently used method is using a bacteriologic loop calibrated which deliver 0.02 or 0.001 ml for culture followed by counting the number of colonies that grow [8].

It is therefore very crucial to have a clear understanding of prevalence of significant bacteriuria among children, indications for diagnostic tests and the appropriate use of antimicrobial agents in the management of children with urinary tract infection.

Materials and Methods

Description of Study Area

Nnamdi Azikiwe University Teaching Hospital (NAUTH), located in Nnewi, is a tertiary health care facility that serves as a referral centre for Anambra and neighboring States in Nigeria.

Study Design

This is a cross-sectional study among attendees of the children out-patients clinic of Nnamdi Azikiwe University Teaching Hospital, Nigeria.

Inclusion Criteria

Children aged 0 to16 yrs. without the exclusion criteria

Attendance to children outpatient clinics of NAUTH.

Exclusion Criteria

Any guardian or parent who did not answer the questionnaire was excluded from the research.

Children with history of antibiotic use less than 7 days before collection of samples were excluded from the research.

Urine Samples

Mid stream urine samples were collected from the participants' aseptically using sterile container.

Samples Processing

The samples were processed using standard microbiological procedures [9].

Culture

A calibrated sterile wire loop that delivered 0.02 ml urine was used and a loopful of a well mixed urine sample was inoculated on Cysteine Lactose Electrolyte Deficient (CLED) Medium (from Oxoid Laboratory, UK.). All plates were incubated at 37°C aerobically for 24 hrs. and the total aerobic count was taken. A significant bacteria count was taken as any count equal to or greater 10^5 CFU/mL.

Identification of Isolates

Identification of isolated bacteria was done by gram staining the isolates followed by microscopic examination of the stained slides, motility test, catalase, oxidase and further identification using API biochemical Test Kits by Biomerieux ® sa.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using Clinical Laboratory Standard Institute (CLSI) standard [10]. The antimicrobial agents used were: Cefotaxime (CFX) 30 µg, Ceftriaxone (CEF) 30 µg, Co-amoxiclav (AXC) 30 µg, Ciprofloxacin (CIP) 5 µg, Imipenem (IMP) 10 µg, Erythromycin (ERY) 5 µg, Cotrimoxazole (COT) 25 µg, Tetracycline (TET) 25 µg (all from Oxoid Laboratories, UK). All plates were incubated for 18-24 hrs. at 37°C in air. Interpretation was carried out according to the CLSI criteria. Appropriate controls were used.

Statistical analysis

Un-paired T-test procedure using Microsoft excel was used for the analysis of data received. P- value greater than 0.05 ($P > 0.05$) was interpreted as having no significant difference.

Results

The significant bacteriuria among 300 children attending out patient clinic at Nnamdi Azikiwe University Teaching Hospital, who met the inclusion criteria of the study were examined. Out of the sampled number, twenty-four children had significant bacteriuria; sixteen (66.7%) were from female participants while eight (33.3%) were from male participants. This resulted to 8% prevalence among the children studied. The distribution of significant bacteriuria which were identified using API Staph and Rapid ID 32E for Gram-positive and Gram-negative organisms respectively are as follows: *E. coli* 6 (25%), *Pseudomonas* 1 (4.17%), *Klebsiella pneumoniae* 5 (20.83%), *Klebsiella oxytoca* 1 (4.17%), *Staphylococcus aureus* 7 (29.17%), *Staphylococcus xylosus* 3 (12.5%), and *Staphylococcus* chromgenes 1 (4.17%) [Table-1]. Urine microscopy showed that 24 specimens had significant bacteriuria [Table-2]. The only *Pseudomonas* isolated expressed resistance to all the third generation Cephalosporin and was susceptible to Imipenem [Table-3]. Most of the isolates of *Staphylococcus aureus* expressed multi-drug resistance across the antimicrobial agents used but was 100% susceptible to Imipenem [Table-4].

Table 1- The distribution of significant bacteriuria among both genders with their respective clinical manifestations

Symptoms	Boys (n=150)		Girls (n=150)	
	Isolated Org.	Patient's age	Isolated Org.	Patient's age
Recurrent fever (>38°C)	*E.coli*	11 yrs.	*S.xylosus*	12 yrs. & 3 yrs.
	E.coli	16 yrs.	*S.aureus*	6 yrs.
	E.coli	7 yrs.	*S.chromogens*	10 yrs.
Swollen leg with fever (>35°C)	*Pseudomonas*	14 yrs.	-	-
Diarrhoea and vomiting with fever	*K.pneumoniae*	2 yrs.	*E.coli*	2 yrs.
			E.coli	3 yrs.
			E.coli	1 yr
Vaginal irritation and Discharge	-	-	*S.aureus*	5 yrs.
	-	-	*S.xylosus*	16 yrs.
	-	-	*S.aureus*	14 yrs.
Catarrh, cold, cough and High body temperature	*S.aureus*	4 yrs.	*S.aureus*	6 yrs.
	K.pneumoniae	14 yrs.	*S.aureus*	8 yrs.
			S.aureus	10 yrs.
Symptoms of malaria with high temperature	*K.oxytoca*	8 yrs.	*K.pneumoniae*	10 yrs.
			K.pneumoniae	17 yrs.
			K.pneumoniae	9 yrs.
Total	8		16	
($p > 0.05$)				

Table 2- Results of Microscopic Examinations of Urine Specimens

Microscopic findings	Significant Bacteriuria Culture after 24 hrs. incubation	Insignificant Bacteriuria Culture after 24 hrs. incubation
Significant Pyuria (> 5 HPF) in a Centrifuged urine	12	3
Insignificant Pyuria (< 5 HPF) in a Centrifuged urine	9	3
Bacteriuria and Significant Pyuria (> 5 HPF) in a Centrifuged urine	2	1
Bacteriuria and Insignificant Pyuria (< 5 HPF) in a Centrifuged urine	1	0
TOTAL	24	7

Table 3- Antimicrobial Susceptibility Pattern of Significant Gram negative Bacteria

Bacteria	Suscep. Pattern	IMP	CFX	AXC	ERY	CIP	COT	TET	CEF
	S (%)	100	20	53	0	43	0	56	20
E.coli (6)	I (%)	0	23	20	20	33	0	12	23
	R (%)	0	57	27	80	24	100	32	57
	S (%)	100	17	10	0	0	0	0	0
K.oxytoca (1)	I (%)	0	0	76	0	88	0	66	0
	R (%)	0	83	14	100	12	100	34	100
	S (%)	100	45	15	0	45	35	25	45
K.pneumoniae (5)	I (%)	0	16	15	25	45	15	45	0
	R (%)	0	39	70	75	10	50	30	55
	S (%)	100	0	0	0	0	0	0	0
Pseudomonas (1)	I (%)	0	0	0	16	78	0	0	17
	R (%)	0	100	100	84	22	100	100	83

S: Susceptible; I: Intermediate; R: Resistant; IMP: Imipenem (10µg); CFX: Cefotaxime (30µg); AXC: Co-amoxiclav (30µg); ERY: Erythromycin (5µg); CIP: Ciprofloxacin (5µg); COT: Cotrimoxazole (25µg); TET: Tetracycline (25µg); CEF: Ceftriaxone (30µg).

Table 4- Antimicrobial Susceptibility Pattern of Significant Gram positive Bacteria

Bacteria	Suscep. Pattern	IMP	CFX	AXC	ERY	CIP	COT	TET	CEF
	S (%)	100	18	14	52	38	12	43	52
S.aureus (7)	I (%)	0	52	43	28	28	14	42	30
	R (%)	0	30	43	20	34	74	15	18
	S (%)	100	20	0	25	0	0	85	16
S.chromogens (1)	I (%)	0	80	0	75	15	10	15	84
	R (%)	0	0	100	0	85	90	0	0
	S (%)	100	70	30	0	33	43	37	23
S.xylosus (3)	I (%)	0	30	43	76	64	13	30	32
	R (%)	0	0	27	24	0	44	33	45

S: Susceptible; I: Intermediate; R: Resistant; IMP: Imipenem (10µg); CFX: Cefotaxime (30µg); AXC: Co-amoxiclav (30µg); ERY: Erythromycin (5µg); CIP: Ciprofloxacin (5µg); COT: Cotrimoxazole (25µg); TET: Tetracycline (25µg); CEF: Ceftriaxone (30µg).

Discussion

From our study, out of 300 patients that participated; 24 had significant bacteriuria giving a prevalence of 8%. Compared to other findings, the rate of UTI among children in the Netherlands were found to be 1.2% from a cross sectional study by Harmsen, et al [11]. In Nairobi, Kenya 13.3% rate of UTI among hospitalized children were reported by Okwara, et al [12]. Ibekwe, et al [13] reported 3% prevalence rate in Abakiliki South Eastern Nigeria. Aiyegoro, et al [14] reported 11.96% among children and adolescents in Ile-ife South-West Nigeria. Extensive health care talk given regularly to school children by highly qualified health personnel could attribute for low incidence of UTI as reported by researchers in that region [11-13].

The most predominant organism from this study was *Staphylococcus aureus* and was responsible for 29.2% cases as shown in [Table-1]. This was followed by *Escherichia coli* (25%), *Klebsiella pneumoniae* (20.8%), *Staphylococcus xylosus* (12.5%), *Pseudomonas* (4.2%), *Staphylococcus chromogenes* (4.2%) and *Klebsiella oxytoca* (4.2%). This finding is similar to the report of Ibekwe, et al [13]. This study showed that there was high incidence of UTI among the female gender (66.67%) than the male gender (33.33%). This could be attributed to the peculiarity of the study area. Some predisposing factors such as; uncircumcision that predisposes young males to UTI was not observed as most male children were circumcised. This also could be responsible for the low prevalence of significant bacteriuria in males observed in this study. This study therefore justifies work done by Schoen, et al [15]. Among the female participants, predisposing factors such as sexual activity reported by Hooton, et al [16], Weir, et al [17], Nguyen, et al [18] Finer and Landau [19], were observed in 1.33% of the patients with UTI. Other predisposing factors responsible for high incidence among the female gender are: close proximity of the female urethral meatus to the anus [20], alternations in vaginal microflora which play a role in encouraging vaginal colonization by coliforms and incomplete voiding of urine among school girls [21]. This study showed that the model class of UTI was 7 yrs. old and the median class was 8 yrs. old. The symptom with the highest occurrence was recurrent fever (body temp ≥ 38°C) 29.2%. The association between recurrent fever and UTI in children has been reported by researchers, like Aiyegoro, et al [14]. It is advised that children especially those less than 5 yrs. of age with recurrent fever (body temperature ≥ 38°C) should be screened for UTI. This is because they may lack the ability to explain their symptoms to either their parents or caregivers.

The use of either urinalysis results or microscopic findings as an index factor for UTI diagnosis can lead to under treatment or over treatment of patients. Two *Staphylococcus xylosus* were isolated from 8 children with only protein in their urine. This organism has been attributed to cause acute pyelonephritis if left untreated. It is also well known to cause UTI in immuno-competent children [22]. It was also noticed that the urinalysis reaction had an association with the time the urine was voided and when the urinalysis test was done. The use of significant pyuria as an index factor for diagnosis of urinary tract infections in patient is not reliable. The microscopic examination of the 300 Urine samples in [Table-2] showed that 15 samples were seen with significant pyuria (pus cells greater than 5 cells per hpf). Out of these, 12 samples had significant bacteriuria after 24 hrs. culture incubation, 3 had insignificant bacteriuria. Out of the 3 samples with insignificant bacteriuria, 2 of the patients showed symptoms suggestive of infection during their clinical presentation. Insignificant pyuria (pus cells less than 5 cells per hpf) were also seen in 12 samples. 9 out of the 12 had significant bacteriuria and 3 with insignificant bacteriuria after 24 hrs. culture incubation. Bacteriuria and significant pyuria were also seen in 3 samples. There was 1 sample with both bacteriuria and significant pyuria but insignificant bacteriuria after 24 hrs. culture incubation. The patient from whom the sample was collected presented with heavy vaginal discharge and irritation. Therefore, from the microscopic findings of this work, it can be suggested that significant pyuria is not a reliable index of urinary tract infection and this is in consonance with Okafor and Okoro findings in their research work at Enugu, Nigeria in 1999 [23]. Sterile pyuria can be caused by many factors such as improper treatment of bacterial urinary tract infection, bacterial UTI with fastidious organisms, Chlamidial urethritis, contamination by vaginal leucocytes, `false` negative urine cultures due to contamination of midstream urine sample with antiseptic and others factors. Fortunately, the issue of false negative urine culture due to contamination of urine samples with antiseptic was meticulously prevented using sterile container for sample collection. Large numbers of microorganisms were isolated from the female patients with the highest bacterial count of 1.1×10^7 colony forming unit (CFU) per mL and the lowest bacterial count being 1.2×10^6 colony forming unit (CFU) per mL. The bacterial counts for both gender ranged from 7.0×10^5 colony forming unit (CFU) per mL to 1.1×10^7 colony forming

unit (CFU) per mL after a 24 hrs. culture incubation using a sterile loop of 0.02 ml.

Conclusion

Since urinary tract infection may be asymptomatic in most cases (as this study has shown), it is therefore suggested that routine screening of patient with unexplained source of fever be done for urinary tract infection and the appropriate antimicrobials administered after sensitivity tests have been carried out in order to prevent the cases of becoming symptomatic later with resultant renal damage. Increase in drug resistance resulting from inappropriate usage of antimicrobials consequently leads to selection pressure. There is the need for government and associated health agencies to curb out strategies to monitor, regulate the use and distribution of antibiotics which will help to reduce the incidence of drug resistance among uropathogens to the readily prescribed antibiotics.

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References

[1] Chang S.L. and Shortlife L.D. (2008) *Pediatr. Clin. Am.,* 53, 379 -400.

[2] Foster R.T. (2008) *Obstet. Gynecol. Clin. North Am.*, 35, 235.

[3] Neal D.E.Jr. (2008) *Urol. Clin. North Am.*, 35, 13.

[4] Freedman A.L. (2002) *Urol. Dis. Am. J.,* 13, 441-490.

[5] Langley J.M., Hanakowski M. and Leblanc J.C. (2001) *Am. J. Infect. Cont.,* 29(2), 94-98.

[6] Schlager T.A., Clark M. and Anderson S. (2001) *Pediatrics*, 108 (4), 7-14.

[7] Rabassa A.I., Shattima D. (2002) *J. Trop. Pediatr.,* 48, 359-361.

[8] Hiroshi O., Yutaka S., Shigenori M., Soichi A., Yukio H. and Sadao K. (2000) *J. Clin. Microbiol.*, 38(8), 2870-2872.

[9] Murray P.R., Rosenthal K.S. and Pfaller M.A. (2007) *Manual of Clinical Microbiology*, 9th ed., ASM Press, 112.

[10] Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing (2011) *Twenty-First Informational Supplement*, 31(1), 40-60.

[11] Harmsen M., Wensing M., Braspenning J.C.C., Wolters R.J., Van der Wouden J.C. and Grol R.P.M. (2007) *BMC Fam. Pract.,* 8(1), 9.

[12] Okwara F.N., Obimbo E.M., Wafuala E.M. and Murila F.V., (2004) *East African Med. J.,* 81(1), 47-51.

[13] Ibekwe R.C., Ibekwe M.U. and Muoneke V.U. (2012) *Ann. Med. Health Sci. Res.,* 2(1), 29-32.

[14] Aiyegoro O.A., Igbinosa O.O., Ogunwonyi I.N., Odjadjere E.E., Igbinosa O.E. and Okoh A.I. (2007) *African J. Microbiol. Res.*, 10, 13-19.

[15] Schoen E.J., Colby C.J. and Ray G.T. (2000) *Pediatrics*, 105 (41), 789-793.

[16] Hooton T.M. and Stamm W.E. (1997) *Infect Dis. Clin. North Am.,* 11, 551-581.

[17] Weir M. and Brien J. (2000) *Adolsec. Med.*, 11(2), 293-313.

[18] Nguyenn H. and Weir M. (2002) *South Med. J.,* 95(8), 867-869.

[19] Finer G. and Landau D. (2004) *Lancet Infect. Dis.*, 4(10), 631-635.

[20] Lipsky B.A. (1990) *Ann. Int. Med.,* 110, 138-150.

[21] Mond G.C., Grunneberg R.N. and Smellie R.N. (1970) *Br. Med. J.,* 1, 602-605.

[22] Freeman J., Goldmann D.A. and Smith N.E. (1990) *N. Eng. J. Med.*, 323, 301-308.

[23] Okafor H.V., Okoro B.A. and Ibe B.C. (1993) *Nig. J. Paediatr.,* 20, 84-88.