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ASYMPTOMATIC BACTERIURIA IN HEALTHY PRIMARY SCHOOL CHILDREN IN ENUGU, NIGERIA

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Abstract: **Asymptomatic bacteriuria (ASB) is common although the prevalence varies widely with age, gender and the presence or absence of genitourinary abnormalities. ASB has been reported to be associated with an increased risk of symptomatic urinary tract infection (UTI) especially in the presence of risk factors and may actually represent the beginning of symptomatic UTI. This study therefore sought to determine the burden of ASB and sensitivity pattern of isolates in primary school children in Enugu.**

Methodology: **This was a cross-sectional descriptive survey involving apparently healthy primary school children aged 6 to 12 years. A pre-tested, care-giver administered questionnaire was used to obtain information about the participants including age, sex, history of fever and antibiotic usage in the two weeks preceding the study. Following a clinical examination, a sample of spot mid-stream urine was collected from each participant for dipstick urinalysis and urine microscopy and culture.**

Results: **Out of the 400 children, 175 (43.75%) were males and 225 (56.25%) were females. The mean age of the children was 10.13 ± 1.81 years.14.25% (57/400) of children had ASB, with gender specific prevalence of 13.7% (24/175) and 14.7% (33/225) for males and females respectively. The prevalence of ASB was higher among the early adolescents (14.4%) and lower in the pre-adolescents (13.6%). *Escherichia coli* (43.8%) and *Staphylococcus aureus* (22.8%) were the most common organisms isolated.**

Conclusion: **The prevalence of ASB is high in primary school children in Enugu, higher in females with *Escherichia coli* as the commonest bacterial isolate. Routine evaluation of these children for bacteriuria is recommended.**

Keywords: **Asymptomatic bacteriuria, Primary school children, Prevalence.**

1. INTRODUCTION

Infection of the urinary tract is among the most common bacterial infections in humans, both in community or hospital settings and have been reported in all age groups and genders.[1] Urinary tract infection (UTI) could be either symptomatic or asymptomatic disease that involves the genito-urinary system.[1] Asymptomatic bacteriuria is defined as significant bacterial count in the urine, usually 10^5 or more colony forming units (cfu) per milliliter in a child without symptoms referable to urinary tracts.[2] High prevalence of asymptomatic bacteriuria is largely due to poor hygiene and sharing of towels and other clothings with older children and adults.[3] Poor toilet sanitation and maintenance coupled with non-availability of water to clean the toilets in primary schools are common in developing countries.[4] ASB could lead to symptomatic UTI especially in the presence of risk factors such as uretheral reflux disease which may cause renal parenchymal infections.[5] This renal parenchymal infection (pyelonephritis) can lead to renal scarring which is the prelude to chronic morbidities associated with urinary infections, such as hypertension, and chronic kidney disease.[5,6] ASB in a

child can also be an indication of an underlying structural or functional urinary abnormality which may require specific therapy to prevent reoccurrence of infection and progression to renal damage.[7] Asymptomatic bacteriuria is associated with an increased risk of symptomatic UTI and may precede symptomatic UTI.[8] Hence, there is need for early detection of this silent infection for prompt referral and intervention. This becomes important because the costs of managing renal diseases are exorbitant and the facilities for renal replacement therapy are very limited and generally not affordable especially in Sub-Saharan Africa.

2. SUBJECTS AND METHODS

Study Design and Population:

This was a cross-sectional and descriptive study conducted in Enugu South local government area of Enugu State, Nigeria. There are 110 officially registered primary schools in the study area, comprising 32 public and 78 private schools with a total population 22,375 children comprising 11,053 males and 11,322 females[14] with male to female ratio of approximately 1:1. The subjects were children aged 6 to 12 years.

Sampling Method:

Multistage sampling method was used in this study. The total number of primary schools selected was achieved by using a proportionate sampling outcome of 10%.[15] The primary schools were then stratified proportionately into public and private schools. Based on a ratio of 1:2.5, a simple random sampling was used to select three schools from the public schools and eight from the private schools.

The sample size of 400 was allocated proportionately to the schools selected using the Neymann proportional allocation formula.[16]

Each of the 11 selected schools had 6 classes: classes 1 to 6. The proportional allocation was applied again to get the sample size for each class.

All the classes from the selected schools had 2 arms. An arm was chosen by simple random sampling method to represent each class. The ratio of male to female was approximately 1:1 in all the selected classes; hence there was no reason to stratify them into male and female. Then, the serial numbers in the register was used to select the pupils randomly with the aid of computer generated table of random numbers.

Ethical Approval and Consent:

Ethical approval was obtained from the Health Research and Ethics Committee of the University of Nigeria Teaching Hospital (UNTH).

Permission was also obtained from the Enugu State Universal Basic Education Board (ESUBEB), Enugu State Ministry of Education as well as from various head teachers of the selected primary schools. An informed written consent was obtained from parents/guardians of each selected pupil. Ascent was also obtained from pupils where applicable.

Preliminary Activities:

A female nurse was recruited and repeatedly trained by the researcher until she understood all the procedures of urine sample collection in females.

The selected subjects were then given a detailed explanation on the procedures involved in urine sample collection and the location within the school premises the urine collection will take place.

Physical examination was carried out on the each of the selected subjects. The perineum of each male subject was also examined to rule out abnormal urethral orifice and to note circumcision status and urinary stream.

Data Collection:

A research proforma designed for the study was used to record the information obtained. The subjects were allowed to go home with the research proforma where the parents or guardians helped with filling of the proforma to get relevant demographic information and past medical history of the subjects. The parents or guardians who could not fill the proforma properly were assisted by the researcher through phone calls.

Specimen Collection:

The subjects who met the inclusion criteria were given consent forms and letters to give their parents or guardians, explaining clearly the nature and purpose of the study.

Inside the school compound, two separate screened enclosures were set up; these were the enclosures where the urine collection took place, one for males and the other for females. The researcher and the female nurse (who was also the assistant to the researcher) supervised the proper collection of urine samples in males and females respectively. Urine samples were collected after cleaning the uretheral orifice and perineum of the male and female subjects with sterile swab respectively.

Midstream urine samples were collected in pre-labeled, sterile, boric acid containing bottles without allowing the bottles to come in contact with external genitalia or adjacent skin to avoid contamination. The urine samples collected were stored in a clean container filled with ice packs and then transported to Microbiology laboratory at UNTH for storage in the refrigerator at 4^0C and subsequently processed within 4 hours of collection.[17]

Laboratory Procedures:

Each urine sample was divided into two parts: One for dipstick analysis and the other for microscopy and culture. Dipstick analysis was done using SD UroColor^{TM}10 (Standard Diagnostics, INC Korea) for nitrite, leucocyte esterase and protein. The test strip was immersed in the urine for 2 seconds,[18] such that all reagent pads were covered by urine. Thereafter, excess urine was removed from the strip by tapping the edge of the strip on the rim of the urine container while holding the strip in a horizontal position to prevent interaction from adjacent reagent pads. The colour change after one minute was compared with the colour chart on the container label under good light with the strip horizontally placed to prevent mixing of chemicals if excess urine was still present.

The other urine part was used for microscopy, culture and sensitivity. It was transferred to the Microbiology Laboratory of UNTH for analysis.

Urine microscopy was carried out by centrifuging 10 ml of urine sample at 2000 revolutions per minute (rpm) for 5 minutes. The supernatant was discarded and a drop of the urine deposit was examined under the microscope at high magnification for pus cells, red blood cells, bacteria and casts.

Culture was done employing the quantitative method as described by Guttman and Stokes.[19] Each uncentrifuged urine sample was well mixed and subsequently inoculated unto well dried plates of blood agar and MacConkey agar as described by Uqurhart and Gould,[20] using a calibrated standard wire loop of 2 mm internal diameter which delivers 0.001 mL of urine per loopful. The wire loop was sterilized over a bunsen burner flame before and after use. The culture plates were incubated prior to inoculation to avoid contamination. Using the standard wire-loop, urine sample was collected from the well mixed specimen and streaked well on to the well dried freshly prepared blood agar and MacConkey plates. The plates were incubated aerobically at 37^oC for 24 hours after which the colonies were counted with a colony counter. The number of colony forming units (CFUs) was multiplied by 1000 to determine the number of microorganisms per mL in the original specimen.

Significant bacteriuria was defined as pure growth of $\geq 10^5$ colony forming units per mL from midstream urine sample. Growth less than 10^5 CFU/mL from the midstream urine sample were regarded as insignificant.[20]

In cases with significant bacteriuria, the bacterial isolates were identified based on colony morphology characteristics, Gram stain reaction and biochemical test using standard techniques (as defined by Murray *et al*[21]). For example, *Escherichia coli* have opaque yellow colonies slightly deeper at the centre on MacConkey agar (lactose fermenter), are motile and negative and positive to indole and vorges procar tests respectively. Klebsiella species have large mucoid yellow or whitish yellow colonies are non-motile and citrate positive while *Staphylococcus aureus* have tiny white and raised colonies, are positive to catalase, DNase and Coagulase tests.

Antibiotic sensitivity pattern of the significant isolates were determined by using the disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards,[22] using diagnostic sensitivity test agar (International Diagnostic Group PLC, Topley House, Bury Lancashire, BL9 6AU, UK) and antibiotic multidiscs (Abtek Biological Limited) with the following antibacterial agents: gentamicin 10mcg, nitrofurantoin 200mcg, cotrimoxazole 25mcg, amoxicillin 25mcg, augmentin (amoxicillin-clavulanate) 30mcg, ofloxacin 5mcg. Others were ciprofloxacin 5mcg, cefuroxime 30mcg, levofloxacin 5mcg and ceftazidime 30mcg.

A straight wire loop was used to pick a small portion of colony under review and seed it on different parts of the Mueller-Hinton agar. Then the seeded organism on the surface of Mueller-Hinton agar was spread using a sterile swab stick. The needed discs were placed onto the Mueller-Hinton agar surface in such a way that they were not clustered together and were then incubated aerobically for 24 hours. After incubation, the diameter of the zone of inhibition was measured and compared with a zone diameter interpretative chart to determine the sensitivity of the isolates to the antibiotics. *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 35218) were employed in all tests as control.

The subjects with asymptomatic bacteriuria were subsequently referred appropriately for follow up.

Data Handling and Statistical Analysis:

The data obtained was recorded on the study proforma and entered into the computer and analyzed using the Software Package for Social Science (SPSS) version 18 for Windows. Data collated were summarized using frequency, percentages, means and standard deviations.

Association between categorical variables was analyzed using chi-square and logistic regression and statistically significant result was attained wherever a p-value was less than the significance level of 0.05.

3. RESULTS

Demographic Characteristics of the Study Population:

A total of 400 apparently healthy children were enrolled into the study between April and July 2015. One hundred and seventy five (44%) were males. The age range was 6 to 12 years with a mean of 10.13 ± 1.81 years. Generally, there was female gender predominance across all age groups except for the 6 year olds but this was not statistically significant. (χ^2 = 2.551, p = 0.863). Table I shows the demographic characteristics of the study population.

Table I: Demographic characteristics of the study population.

Age (years)	Male n (%)	Female n (%)	Total n (%)
6	9 (5.2)	8 (3.5)	17 (4.3)
7	9 (5.2)	16 (7.1)	25 (6.3)
8	15 (8.5)	24 (10.7)	39 (9.8)
9	25 (14.3)	29 (12.9)	54 (13.5)
10	34 (19.4)	42 (18.7)	76 (19.0)
11	25 (14.3)	26 (11.5)	51 (12.6)
12	58 (33.1)	80 (35.6)	138 (34.5)
Total	175 (100.0)	225 (100.0)	400 (100.0)

χ^2 = 2.551, p = 0.863

Prevalence of Asymptomatic Bacteriuria (ASB) and Colony Counts.

Seventy (17%) of the 400 urine samples had bacterial growth but only 57 (14.25%) had significant counts ($\geq 10^5$ colony forming units (CFU)/ml) (Table II).

Table II: Urine colony counts in the bacteriuric subjects.

Colony count (CFU/ml)	Frequency	Percent
$\geq 10^5$ (significant bacteriuria)	57	14.25
10^4 - 10^5	4	1.00
$<10^4$	9	2.25
Total	70	17.00

The Gender and Age Distribution of Children with ASB:

Of the 57 children with ASB, 33 (57.9%) were females and 24 (42.1%) males with a male-female ratio of 1:1.4. The gender specific prevalence of ASB in females was 14.7% (33/225) while that of males was 13.7% (24/175). The prevalence of ASB did not differ significantly between females and males. (χ^2 = 0.073, p = 0.787).

The prevalence of ASB in the early adolescent group (9 to 12 years) was 14.4% (46/319) while a prevalence of 13.6% (11/81) occurred in the preadolescent group (6 to 8 years) but the prevalence of ASB between these two age groups was not statistically significant. (χ^2 = 1.514, p = 0.218).

Bacterial Isolates from Children with ASB:

A total of 57 bacterial isolates were obtained. *Escherichia coli* was the most predominant organism isolated and was responsible for 25/57 (43.8%) of the cases of ASB. This was followed by *Staphylococcus aureus* 13/57 (22.8%), *Klebsiella aerogenes* 9/57 (15.8%) and *Staphylococcus epidermidis* 4/57 (7%). Others were *Pseudomonas aeroginosa* and *Streptococcus faecalis* which accounted for 2/57 (3.5%) each while *Proteus mirabilis* and Enterobacter accounted for 1/57 (1.8%) of the isolates each.

Bacterial Isolates and Gender:

The gender specific prevalence of bacterial isolates in males and females is as in figure 1, with *Escherichia coli* predominating in both sexes.

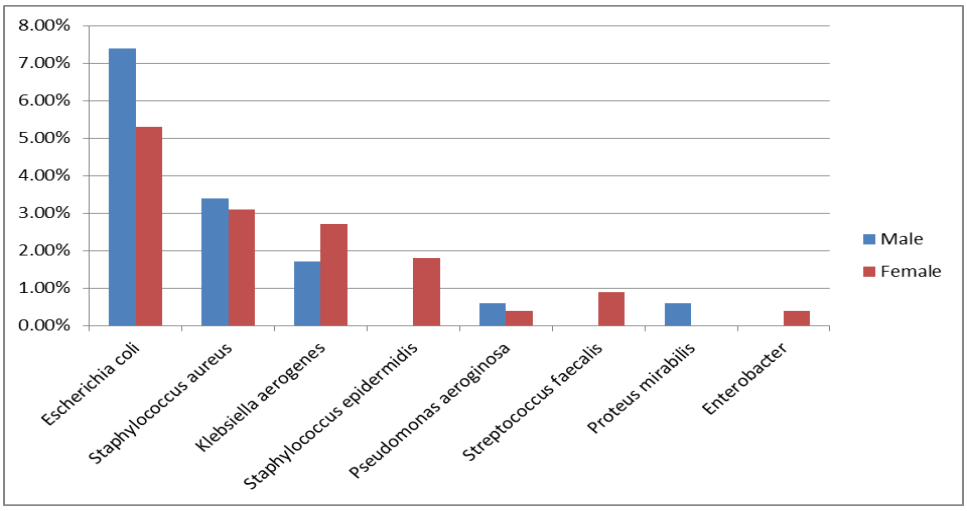

Figure l: Bacterial isolates and Gender

Distribution of Bacterial Isolates among Pre-adolescents and Adolescents:

A total of 57 of the 400 subjects (14.25%) had uropathogens. Of this number, 11 of the 57 (19.30%) were pre-adolescents. This represents 11 of the 81 (13.58%) of the total pre-adolescent population with uropathogens. Similarly, 46 of the 57 (80.70%) were adolescents. This represents 46 of the 319 (14.42%) of the total adolescent population with uropathogens.

Escherichia coli was isolated from 25 urine samples, 80% (20/25) from adolescents, compared to 20% (5/25) from pre-adolescents. *Staphylococcus aureus* was seen in 13 urine samples, 85% (11/13) from adolescents, compared to 15% (2/13) from pre-adolescents. *Klebsiella aerogenes* was seen in 9 urine samples, 89% (8/9) from adolescents, compared to 11% (1/9) from pre-adolescents. *Staphylococcus epidermidis* was seen in 4 urine samples, 75% (3/4) from adolescents, compared to 25% (1/4). *Pseudomonas aeroginosa* was seen in 2 urine samples, all from the pre-adolescents. *Streptococcus faecalis* was seen in 2 urine samples, all from the adolescents. *Proteus mirabilis* and Enterobacter were seen in 1 urine sample each, all from the adolescents. (Table III).

Table III: Distribution of Bacterial Isolates among Pre-adolescents and Adolescents

Bacterial Isolates	Age (years)		
	Pre-adolescents: 6-8 n (%)	Adolescents: 9-12 n (%)	Total
Escherichia coli	5(20)	20(80)	25
Staphylococcus aureus	2(15)	11(85)	13
Klebsiella aerogenes	1(11)	8(89)	9
Staphylococcus epidermidis	1(25)	3(75)	4
Pseudomonas aeroginosa	2(100)	0(0)	2
Streptococcus faecalis	0(0)	2(100)	2
Proteus mirabilis	0(0)	1(100)	1
Enterobacter	0(0)	1(100)	1
	11	46	57

Antibiotics Sensitivity Pattern:

Of the 25 *Escherichia coli* isolated, 24(96%) of them were sensitive to ciprofloxacin, followed by levofloxacin and ceftazidime to which 22(88%) isolates each were sensitive. Of the 13 *Staphylococcus aureus* isolates, 12(92.3%) each were sensitive to gentamicin and clavulanate potentiated amoxicillin, followed by ciprofloxacin, levofloxacin and ofloxacin to which 11(84.6%) isolates each were sensitive. Of the 9 *Klebsiella aerogenes* isolates, all were sensitive to ciprofloxacin, levofloxacin, ofloxacin and gentamicin. Others were as tabulated on Table IV.

Table IV: Bacterial isolates and their antibiotic sensitivity patterns

Isolated Pathogens (n)	CIP	LEV	OFL	GEN	CAZ	CRX	AUG	AMX	COT	NIT
Escherichia coli (25)	24(96.0%)	22(88.0%)	21(84.0%)	20(80.0%)	22(88.0%)	16(64.0%)	6(24.0%)	5(20.0%)	7(28.0%)	5(20.0%)
Staphylococcus aureus (13)	11(84.6%)	11(84.6%)	11(84.6%)	12(92.3%)	7(53.8%)	6(46.2%)	12(92.3%)	8(61.5%)	8(61.5%)	6(46.2%)
Klebsiella aerogenes (9)	9(100%)	9(100%)	9(100%)	9(100%)	5(55.6%)	6(66.7%)	3(33.3%)	3(33.3%)	2(22.2%)	3(33.3%)
Staphylococcus epidermidis (4)	4(100%)	3(75.0%)	4(100%)	4(100%)	4(100%)	1(25.0%)	4(100%)	4(100%)	1(25.0%)	3(75.0%)
Pseudomonas aeruginosa (2)	2(100%)	2(100%)	2(100%)	2(100%)	2(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Streptococcus faecalis (2)	1(50.0%)	2(100%)	1(50.0%)	0(0%)	1(50.0%)	1(50.0%)	2(100%)	1(50.0%)	0(0%)	0(0%)
Proteus mirabilis (1)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)
Enterobacter (1)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)
All pathogens (57)	53(93.0%)	51(89.5%)	50(87.7%)	49(86.0%)	43(75.4%)	32(56.1%)	28(49.1%)	22(38.6%)	20(35.1%)	18(31.6%)

CIP = Ciprofloxacin OFL = Ofloxacin CAZ = Ceftazidime AUG = Augumentin COT = Cotrimoxazole

LEV = Levofloxacin GEN = Gentamicin CRX = Cefuroxime AMX = Amoxicillin NIT = Nitrofurantoin

4. DISCUSSION

The prevalence of 14.25% for ASB obtained in this study is similar to what was observed in other studies in Nigeria.[23,24,25] Mbakwem-Aniebo *et al*[23] in Port Harcourt, Nigeria documented a prevalence of 14.5% amongst primary school children, Akor *et al*[24] studied primary school children in Jos and found a prevalence of 14% while Onifade *et al*[25] in Ibadan reported a prevalence of 15.5% which are all comparable with the present study.

In contradistinction, some studies have reported ASB prevalence rates much higher than that obtained in the present study. Jeena *et al*[26] in Durban, South Africa in 1996 and Modarres *et al*[27] in Iran in 1997 found prevalence of 26% and 25.8% respectively in subjects under 12 years of age. Their high prevalence was due to subject selection as their subjects included uncircumcised males unlike the present study where all the males were circumcised. In addition, Jeena *et al*[26] and Modarres *et al*[27] included large number of young infants who are known to have a higher predisposition to ASB because of presence of urinary tract abnormalities in some of them.[17] Salem *et al*[28] in 2009 documented a prevalence rate of 30% in Egyptian school children which was also higher than the prevalence rate from the present study. The fact that Salem *et al*[28] studied children with type 1 diabetes could have also contributed to the high values they found. Children with diabetes mellitus tend to have higher rates of ASB because of reduced immunity and urines with high sugar content could be a culture medium.[29] While this present study was carried out in a community, those of Salem *et al*[28] and Jeena *et al*[26] were hospital-based studies because those children were already ill. Such children are considered to have a higher risk of ASB.[30] Sample contamination may also be more in them.[30]

In Nigeria, reports of higher ASB rates include 30% by Azubuike *et al*[13] among primary school children in Awka in 1994 and 48% by Alo *et al*[31] in rural primary school children in Ebonyi State in 2012. Azubuike *et al*[13] enrolled a relatively lower sample size of 200, compared to the 400 in the current study. The sample size of 200 in the study by Azubuike *et al*[13] did not represent the appropriate percentage of the total population (33,125 pupils) of the primary school children in Awka[15] and one primary school was used in their study which could lead to poor distribution of subjects and may perhaps be responsible for their high ASB prevalence. Alo *et al*[31] used a diagnostic cut-off of $>10^4$ CFU/ml instead of $>10^5$ CFU/ml as their definition of significant bacteriuria using midstream urine and this could also be one of the reasons for the high ASB rate. Moreover, this high ASB rate of 30% and 48% documented by Azubuike *et al*[13] and Alo *et al*[31] respectively could be attributed to the fact that the studies were carried out among children in rural areas who are known

to have low levels of hygiene and poor health consciousness and Awka was considered rural two decades ago.[13] Although, most of the studies did not indicate the time interval between urine collection and analysis, time lag between the two processes may also account for higher ASB rates in some of the studies because of probable bacteria proliferation.[32]

On the other hand, some other studies documented ASB prevalence rates much lower than that observed in the present study. Joseph *et al*[33] in India in 1989 and Yayli *et al*[34] in Turkey in 2003 documented prevalence rates of 0.12% and 0.37% respectively. It was noted that Joseph *et al*[33] and Yayli *et al*[34] screened a much larger population of 2447 and 10,289 school children respectively, compared to 400 school children in the present study. While it could be argued that the improved level of hygiene in developed countries such as Turkey may be contributory to low ASB rates among the participants in Yayli *et al*[34] study, the same cannot be said for India which is a developing country.

In Nigeria, Elegbe *et al*[35] in Ile-ife in 1987 and Akinkugbe *et al*[36] in Ibadan in 1988 documented prevalence rates of 5% and 4.7% respectively among school children which were quite lower than the present study. These low rates were attributed to the greater attention paid to health education in their school curriculum where all the rudiments of environmental and personal hygiene were taught.[35,36]

There was no statistically significant difference in the prevalence of ASB across the two age groups (pre-adolescent and early adolescent) in this study. Nonetheless, the higher rate of ASB was found in the 9-12 years group (the early adolescent age group). This observation is similar to the findings of Mbakwem-Aniebo *et al*[23] who recorded higher ASB prevalence in the 10-12 years age group. The higher prevalence of ASB in this age group may not be unconnected with the fact that this age group is the early adolescent age and some of the subjects may have been sexually exposed.[37,38] Moreover, education on basic perineal hygiene may not have been properly inculcated in the early adolescent age groups and they, more often than not, will want to do the perineal cleaning themselves unlike the pre-adolescents, where the perineal cleaning are usually done by the parents or older relatives.

In the present study, ASB was commoner in females than in males. The observed trend is in tandem with findings contained in the studies done by Alo *et al*,[31] Sawalha *et al*,[39] Saleh *et al*[40] and Mbakwem-Aniebo *et al*.[23] The female predominance could be attributed to the short female urethra, which is in close proximity to the anus from where it can be easily contaminated by faecal matter, alterations in vaginal microflora which play a role in encouraging vaginal colonization by bacterial pathogens and incomplete voiding of urine among school girls.[27,41] Some predisposing factors such as non-circumcision that predisposes males to ASB[42] were not observed in this study as all male children in this study were circumcised. This may also have contributed to the lower prevalence of ASB in males as seen in this present study. This observation is however in disagreement with results obtained by Wennerstrom *et al*[43] who found higher ASB rates in males compared to females. This male predominance seen in the study by Wennerstrom *et al*[43] was attributed to high incidence of uncircumcised males in their study.

Escherichia coli was the commonest pathogen isolated in this study, followed by *Staphylococcus aureus*, *Klebsiella aerogenes* and *Staphylococcus epidermidis.* The studies done by Oluyemi *et al*[44] in Akure, Ondo State and Mbakwem-Aniebo *et al*[23] in Port Harcourt also reported *Escherichia coli* as the commonest isolates among school children. Similarly, other studies done within the country by Musa-Aissien *et al*,[45] Asinobi *et al*[46] and Dogunro[47] reported *Escherichia coli* as the commonest bacteria isolated in urine. Some studies[39,40,48] carried out outside Nigeria have also reported *Escherichia coli* as the most common bacterial isolate. This reflects the origin of the bacterial pathogen which is usually from the microflora of the intestine and perineum.[49,50] *Escherichia coli* has fimbriae on its surface which contains adhesin at the tip that bind to uroepithelial cells.[6] The adhesion factors diminish the bacteria washout with voiding and permit the bacteria to persist and cause infection.[6]

In contrast, study by Eke and Eke[51] in Port Harcourt reported *Klebsiella* as the commonest bacterial isolate implicated in asymptomatic bacteriuria, (occurring almost in equal proportion with *Escherichia coli* (25.9% versus 25.5%). Similarly, Anochie *et al*[52] found *Klebsiella aerogenes* to be the commonest organism in urinary isolates followed by *Staphylococcus aureus* and *Escherichia coli.* Adeyemo *et al*[47] also reported Klebsiella species as the predominant isolate in both in-patients and out-patients in Ibadan followed by *Escherichia coli*. Muoneke *et al*[53] in Abakaliki also reported Klebsiella as the most predominant uropathogen followed by *Staphylococcus aureus*. Klebsiella is found among malnourished persons and it can also be associated with hospital acquired infection of the urinary tract.[54] Therefore, the implication of the Klebsiella presence is likely to be that these children were malnourished and could have acquired the infection at the hospital.[54] The inference is that it is unlikely to have Klebsiella as the predominant pathogen in the present study because apparently healthy subjects were enrolled and it was a community based study.

In contrast too, other authors have documented different pathogens as main causative agents. Alo *et al*[31] in a rural community in Ebonyi State among school children found *Staphylococcus aureus* and *Klebsiella pneumonia* (11.6%) each. Azubuike *et al*[13] documented *Staphylococcus epidermidis* as the most prominent urothogen among school children, followed by *Escherichia coli*. Human beings harbour Staphylococcus on their skin and this organism is a good indicator of the standard of hygiene.[55] The bacterium is also widely distributed in the environment especially the unhygienic ones.[56] The studies by Alo *et al*[31] and Azubuike *et al*[13] were carried out in a rural community where the subjects were more likely to live in unhygienic environments unlike the present study which was carried out in urban community. This may be the reason why Staphylococcus was the predominant uropathogen isolated in their studies. From the above analysis, it becomes pertinent to continue to monitor the pattern of uropathogens in each locality as the pattern could change depending on the prevailing circumstances.

In this study, *Escherichia coli* and *Staphylococcus aureus* predominated in males compared to females. This observation was consistent with findings by Mbakwem-Aniebo *et al*[23] who also reported predominance of *Escherichia coli* and *Staphylococcus aureus* in males. This may be due to poor personal hygiene by the males. It may also be due to self-instrumentation where some school aged males as a form of abnormal habit, inject water into their urethra while taking bath.[57] However, *Klebsiella aerogenes*, *Staphylococcus epidermidis*, *Staphylococcus faecalis* and Enterobacter predominated in females, compared to males. This may be due to shorter urethra and close proximity of the female urethral meatus to the anus, alterations in vaginal microflora and incomplete voiding of urine in school girls.[27,41]

In this study, the antibiotic sensitivity patterns of the isolates revealed high sensitivity to quinolones (ciprofloxacin, levofloxacin and ofloxacin). Similar sensitivity pattern to the quinolones have been reported by other studies within and outside Nigeria.[55,56,58-62] Gupta *et al*[55] in USA in 1999 reported that resistance to fluoroquinolones was absent among gram-negative pathogens in their study. Aiyegoro *et al*[56] in Ile-Ife, Nigeria in 2007 reported ofloxacin to be the most effective antibiotic against urinary isolates, followed by ciprofloxacin. Similarly, Kolawole *et al*[59] in Ile-Ife in 2009 reported the quinolones (ofloxacin, ciprofloxacin and perfloxacin) to be the most potent of all the antibiotics in their study. Brown *et al*[58] in Ibadan in 2004 documented highest sensitivity to perfloxacin to which over 74% of the urinary isolates from febrile children were sensitive. Musa-Aissien *et al*[45] in Benin in 2003 also documented high sensitivity of most urinary isolates (77%) to ciprofloxacin. In a UK muticenter study,[63] ciprofloxacin was highly active against the uropathogens with sensitivities in the range of 88.6% and 97.7% for most prevalent pathogens including *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia* and *Proteus mirabilis*. The high susceptibility of the urinary pathogens to quinolones may be attributed to the fact that quinolones are relatively new drugs that have not been extensively used, thus discouraging the development of resistance to them by pathogens. Moreover, quinolones have been shown to cause arthropathy in animal studies and this may be extrapolated to children who are still growing.[61] Quinolone tablets are known to have bitter taste and may not be tolerated by children and syrup forms are not yet available.

In this study, gentamicin was active against 86% of the isolates. The high susceptibility of uropathogens to gentamicin in the present study is comparable to that noted in the studies done by Musa-Aissien *et al*[45] in Benin in 2003 and Wammanda *et al*[64] in Zaria in 1999, who found 80% of the isolates to be sensitive to this drug. Similarly, Jeena *et al*[26] in South Africa in 1996, found all gram negative pathogens to be 100% sensitive to gentamicin. The high sensitivity to gentamicin recorded in the present study may be attributable to the fact that this drug, although cheap, is less commonly used especially at home because of its injectable nature. However, lower sensitivity to this drug has been described in the study by Adeyemo *et al*[65] in Ibadan in 1994.

Less than half of the isolates were sensitive to clavulanate potentiated amoxicillin in the present study. This is lower than the 81% sensitivity noted in Benin.[45] However, Brown *et al*[58] found only 22.1% of urinary isolates to be sensitive to this drug. The resistance of urinary pathogens (especially *Escherichia coli*) to this drug implies that clavulanate potentiated amoxicillin may not be very effective in the treatment of children with urinary infections.

Nitrofurantoin, cotrimoxazole and amoxicillin were the least effective antibiotics in this study as more than 65% of the isolates were resistant to them. Okafor *et al*[66] in Enugu documented 68% resistance of all isolates to cotrimoxazole over two decades ago. This is comparable to 65% resistance reported in this study. The high resistance of urinary pathogens to these older drugs seem to have persisted over the years and has been documented by other workers within and outside Nigeria.[47,58,60,67] The practice of self-medication, use of fake and sub-standard drugs including drug abuse may perhaps be responsible for the observed trend.

Our study had some limitations. Children with asymptomatic bacteriuria, particularly those with repeat infections and males would have benefited from imaging studies. This would have helped in determining the presence congenital or structural urogenital abnormalities.

This was limited by the high cost of these investigations.

5. CONCLUSION

In conclusion, ASB remains an important problem in school children. The prevalence of ASB in Enugu South LGA was 14.25% and is commoner in children aged 9-12 years. The commonest isolate in ASB was *Escherichia coli.* Over 90% of isolates in ASB were sensitive to ciprofloxacin but most isolates in ASB demonstrated high in-vitro resistance to commonly used antibiotics such as cotrimoxazole, amoxicillin and clavulanate potentiated amoxicillin.

It was therefore recommended that there is need for regular, periodic monitoring of the pattern of organisms causing asymptomatic bacteriuria and their antibiotic sensitivity pattern in various localities to determine changing trends. Ciprofloxacin, levofloxacin, ofloxacin and gentamicin could be considered for empiric treatment of UTI in Enugu because of their high in-vitro sensitivity by isolates. However, their use should be guided, as much as possible, by antibiotic sensitivity studies.

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CONFLICT OF INTEREST:

No conflict of interest was declared by the authors.

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