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Prevalence of asymptomatic bacteriuria among pre-school children in Nnewi, South-East Nigeria

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Abstract Background: Early diagnosis and management of urinary tract infection (UTI) in young children reduces the risk of renal scarring and chronic renal insufficiency. We determined the prevalence of asymptomatic bacteriuria (ASB) among pre-school children in Nnewi, South-East Nigeria.

Methodology: This was a cross-sectional survey involving apparently healthy nursery school children aged 3-5 years. A pre-tested, care-giver administered questionnaire was used to obtain information about the participants including age, sex, history of fever and antibiotic administration in the two weeks preceding the study. Following a clinical examination, a sample of mid-stream urine was collected from each participant for dipstick urinalysis, and urine microscopy and culture. ASB was defined as the presence of $\geq 10^5$ CFU/ml of urine in a participant who had no symptoms of UTI.

Results: Out of 792 children, 417 (52.3%) were females and 375 (47.4%) were males. The mean age of the children was 4.0 ± 0.7 years. ASB was found in 31 children (4%). The prevalence of ASB in females (7.2%) was significantly higher than in males (0.5%), $p < 0.001$. The highest prevalence of ASB of 5.6% occurred in the 4-year-olds and the lowest of 2.0% occurred in 5 year olds, $p = 0.09$. The commonest bacterial isolates among the ASB cases were *Staphylococcus aureus*, 13 (40.6%); *Streptococcus faecalis*, 9 (28.1%) and *Escherichia coli*, 5 (15.6%).

Conclusion: Asymptomatic bacteriuria is commoner in female pre-school children and *S. aureus* is the commonest bacterial isolate. Routine evaluation of female pre-school children for bacteriuria is recommended.

Key words: Asymptomatic bacteriuria, Pre-school children, Prevalence

Introduction

Infection of the urinary tract is among the commonest causes of diseases in childhood.¹ It affects the entire paediatric age group, and has been shown to be a significant cause of morbidity and long-term complications especially in the tropics and subtropics.² In children, especially the pre-school age group, urinary tract infection (UTI) could be symptomless otherwise known as asymptomatic bacteriuria (ASB).³ When symptoms are present, they are usually vague and non-specific, and may not be referable to the urinary tract.³ It may therefore escape diagnosis by the primary care physician.

Asymptomatic bacteriuria may be followed by symptomatic UTI. Whether symptoms are present or not, renal damage and scarring occur in a number of patients

especially if it occurs before five years of age.³ About 10-35% of children with ASB have vesico-ureteric reflux and other renal abnormalities.³ It has also been shown that there is an association between renal scarring during childhood and increased risk of hypertension and chronic renal insufficiency in later life.⁴ However, early diagnosis of UTI and prompt treatment reduces the risk of renal impairment and prevents deterioration of renal function in such children.³

Urine microscopy/culture is not routinely carried out in the evaluation of apparently healthy children especially in resource-limited settings. This poses a challenge to the identification of children with ASB and further compromises the benefits of timely intervention to prevent possible renal damage. We carried out a cross-sectional survey to determine the prevalence of ASB in a large

population of pre-school children in Nnewi, South-East Nigeria.

Materials and Methods

Study design and population

This was a cross-sectional study conducted in Nnewi town, Anambra State in South-East Nigeria. The population of the town at the time of the study was about 121,065.⁵ The inhabitants of Nnewi are predominantly Ibos and their occupation is mainly trading and subsistence farming, with few public servants. As at the time of this study, the town had four geographical quarters with 49 local government-approved nursery schools all of which were privately owned. The study duration was between February and April, 2004. Eight nursery schools, two from each quarter of the town were randomly selected.

The study population comprised apparently healthy nursery school children aged 3-5 years as at their last birthday, who met the inclusion criteria. Only children who were toilet trained and off nappies were enrolled. Children with fever, body swelling or symptoms suggestive of genitourinary abnormalities were excluded from the study. In addition, malnourished children and those with history of antibiotic use in the preceding two weeks of the study were also excluded.

Permission for the study was obtained from the Ethical Committee of the Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi as well as Nnewi North Local Government School Board. The headmaster/headmistress of each selected school also approved of the study and the project was appropriately explained to the teachers and parents. Informed consent was obtained from the parents/guardians of the children.

Pilot Study

A pilot study was conducted in December, 2003 on 30 pupils randomly selected from a nursery school in one of the quarters of the town. Questionnaires were administered to the parents/guardians of the 30 pupils and were self-completed at home. Another 30 randomly selected pupils had their parents/guardians interviewed using researcher-administered questionnaires. The responses were compared and there was no significant difference between the self-completed responses and the responses from the researcher-administered questionnaires. Based on this, it was concluded that the questionnaire was understood and could easily be completed at home by the care-givers. Data obtained from the pilot study were not included in the main study.

Sample size and Sampling

A minimum sample size of 750 subjects was calculated using the formula for cross-sectional surveys:⁶

$$N = \frac{Z^2 PQ}{\delta^2}$$

Where N = minimum sample size, Z= constant at 95% confidence interval= 1.96, P= Prevalence of ASB from a previous study in South-East Nigeria= 2.1%⁷, Q = (1 - P) = 1 - 0.103 = 0.897, δ =precision allowed=0.05. In consideration of a 10% attrition rate in the pilot study, the sample size was increased to 825. The schools in each quarter of the town were listed and two schools were randomly selected by balloting. In each school selected, the school register was used to stratify the nursery school population into 3, 4 and 5 years age strata, and each age stratum was further divided into male and female sub-groups.

Data Collection

Information obtained with the questionnaires included date of birth/age, sex, history of fever and antibiotic administration in the preceding two weeks. Data collection lasted for an average of 10 days in each school. This period was used for the selection of the subjects, distribution and retrieval of the questionnaires, and collection of urine samples. The pre-tested questionnaire was administered to the parents/guardians of the pupils as they brought or collected their wards from school. For the few of them that could not be directly reached by the researchers, the questionnaires were administered through the child's teacher. The parents/guardians were encouraged to complete the questionnaires at home and to return them the subsequent day. Those who were not literate were assisted with translation by the class teachers before the questionnaires were completed.

A clinical examination was performed on each of the participants with emphasis on anthropometry (height and weight), axillary temperature, general physical examination, and features of renal disease such as facial puffiness, oedema, renal angle tenderness and ballotable kidneys. Axillary temperature of ≥ 37.5 °C was regarded as fever.⁸ Weight was measured using a scale with a sensitivity of ± 50 g. The scale was checked for zero error and standardization was done with standard weight before using it. The values of the weight and height were compared with standards according to the National Centre for Health Statistics charts in order to exclude those who were malnourished.

A sample of mid-stream urine was collected from each participant with the help of the class teacher into previously labeled sterile universal bottle containing boric acid crystals. In females, the class teachers parted the labia during micturition and in all subjects it was ensured that the specimen bottle did not come in contact with the perineum, external genitalia or adjacent skin to avoid contamination. The urine samples were transported to the microbiology laboratory within two hours of collection for immediate analysis. The urine specimens were divided into two aliquots. One aliquot was used for dipstick urinalysis while the second aliquot was used for microscopy and culture.

Laboratory investigations

Dipstick urinalysis

Dipstick urinalysis was done on one aliquot of uncentrifuged urine using multistix 10SG (Bayer incorporations, USA) which is capable of testing for ten different parameters including protein, pH, glucose, specific gravity, leucocytes, urobilinogen, bilirubin, ketones, nitrites and blood.

Urine microscopy

Ten milliliters of urine specimen from each participant was centrifuged in a test tube at 3,000 revolutions per minute (rpm) for five minutes. The supernatant fluid was decanted and the remaining contents were shaken to mix and a wet preparation made with one or two drops of the sediment. The slide was examined under high power objective of a microscope for white blood cells, red blood cells, bacteria debris and casts. The leucocyte count per high power field was taken as the average value from at least four high power fields.

Urine culture, colony count and bacterial identification

Urine culture was carried out by simultaneous plating of blood agar and MacConkey agar plates. Before inoculation of the urine, there was prior incubation of the plates at 37°C for 30 minutes to dry the surface and eliminate contamination. The inoculated plates were incubated aerobically at 37°C for 18-24 hours, after which the colonies were counted using the Kass criteria.⁹ Bacterial identification was done using various methods including the appearance of the colonies, gram staining and standard biochemical methods. The Biochemical methods included triple sugar iron, oxidase, coagulase, urease and indole tests. Gram staining was used to classify the bacteria into gram positives and gram negatives.

Case definitions

The following case definitions were applicable:⁹

- Significant pyuria was defined as the presence of five or more leucocytes per high power field (≥ 5 WBC/hpf).
- Significant bacteriuria was regarded as bacteria counts of $\geq 10^5$ colony forming units per ml (CFU/ml).
- Negative culture was defined as bacteria counts of $< 10^4$ CFU/ml.
- Asymptomatic bacteriuria was said to be present when a urine sample contained $\geq 10^5$ CFU/ml of urine in a participant who had no symptoms of UTI.
- Contaminated sample: culture plates with colony counts of $> 10^4$ but $< 10^5$ CFU/ml growing two or more organisms. For contaminated samples, the urine culture was repeated with a fresh specimen collected from the pupil concerned within 48 hours.

Statistical analysis

Data analysis was carried out using the Statistical Product and Service Solutions (SPSS) version 15. Means (\pm SD) and proportions were used to describe continuous and categorical variables respectively. Differences between proportions were tested using Yates's corrected chi square test or Fisher's exact test. Statistical significance was set at $p < 0.05$.

Results

Age and sex distribution of the children

Table 1 shows the age and sex distribution of the study participants. Out of 825 children enrolled, 33 had inadequate data for analysis leaving a total of 792 children. Out of 792 children, 417 (52.3%) were females and 375 (47.4%) were males. The ages of the children ranged from 3 to 5 years, with a mean age of 4.0 ± 0.7 years. Across all ages, there were more females than males but the difference was not statistically significant, $p=0.62$.

Table 1: Age and Sex distribution of all the children studied

Age (years)	Male n (%)	Female n (%)	Total n (%)
3	108 (28.8)	114 (27.3)	222(28.0)
4	155 (41.3)	165(39.6)	320(40.4)
5	112(29.9)	138(30.1)	250(31.6)
Total	375(100)	417(100)	792(100)

$\chi^2=0.96$, $p=0.62$

Prevalence of asymptomatic bacteriuria and colony counts

The prevalence of ASB and colony counts is shown in Table 2. Thirty two of the 792 urine samples had colony counts $\geq 10^5$ CFU/ml and all qualified as ASB giving a prevalence of 4.0%. Fourteen urine samples had bacterial growth between 10^4 and 10^5 CFU/ml while 62 urine samples had colony count of $< 10^4$ CFU/ml. Six hundred and eighty four urine samples (86.4%) were sterile.

Table 2: Urine colony counts in the study population

Colony count (CFU/ml)	N=792 n (%)
$> 10^5$ (ASB)	32(4.0)
10^4 - 10^5	14(1.8)
$< 10^4$	62(7.8)
Sterile	684 (86.4)

ASB= Asymptomatic bacteriuria, CFU= Colony forming units

Table 3 shows the sex and age distribution of participants with ASB. Of the 32 children with ASB; 30 (93.7%) were females and 2 (6.3%) were males. The prevalence of ASB in females (7.2%) was significantly higher than in males (0.5%), $p < 0.001$. The highest prevalence of ASB of 5.6% occurred in the 4-year-olds

and the lowest of 2.0 % occurred in 5 year olds but the difference in the prevalence of ASB across the ages was not statistically significant as $p=0.09$.

Table 3: Distribution of asymptomatic bacteriuria by age and sex

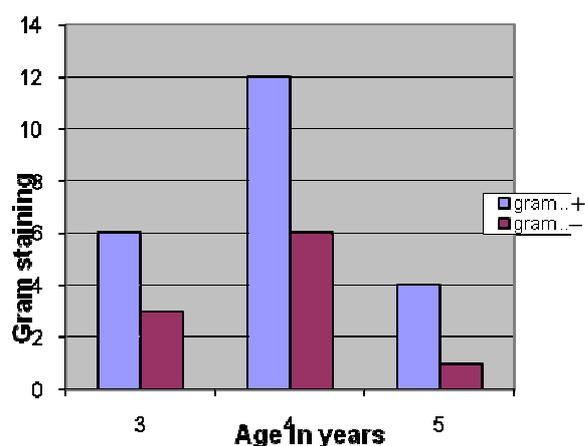
Variable	Asymptomatic bacteriuria		Total	χ^2	p-value
	Yes n (%)	No n (%)			
Sex					
Female	30 (7.2)	387 (92.8)	417	21.1	<0.001
Male	2 (0.5)	373 (99.5)	375		
Total	32	760	792		
Age (years)					
3	9 (0.04)	213 (0.96)	222	4.8	0.09
4	18 (0.06)	302 (0.94)	320		
5	5 (0.02)	245 (0.98)	250		
Total	32	760	792		

Bacterial isolates from children with ASB

Gram positive organisms were the predominant bacterial isolates, responsible for 22 (68.8%) of the cases of ASB while gram negatives were responsible for 10 (31.2%) of the cases. The two (100%) males with ASB had gram-positive organisms while 20 (62.5%) out of the 30 females with ASB had gram positive organisms. The remaining 10 (31.3%) females had gram negative organisms.

Figure 1 shows the Gram staining distribution of the organisms according to age. Gram positive organisms were predominant across all ages, 80% in children aged 5 years, 66.7% in the 4 year olds and 66.7% in the 3 year olds. The difference in the rate of gram positive organisms was not statistically significant across the ages, $\chi^2=4.76$, $p=0.09$.

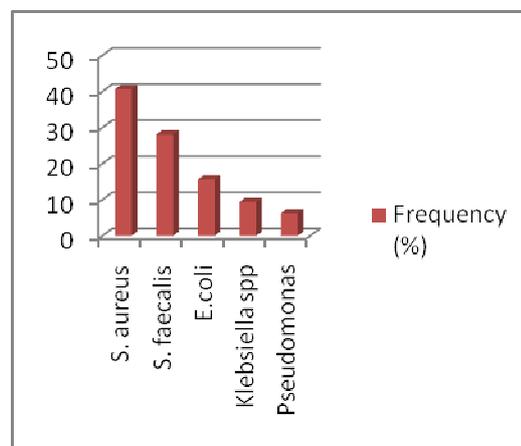
Fig 1: Gram staining distribution of the organisms according to age



The bacterial isolates in the children with ASB is shown in Fig. 2. The commonest organisms were *Staphylococcus aureus*, 13 (40.6%); *Streptococcus faecalis*, 9 (28.1%) and *Escherichia coli*, 5 (15.6%). The two males with ASB had either of *Streptococcus faecalis* and *Staphylococcus aureus* isolated from their samples. The organisms isolated in the females were *Staphylococcus aureus* 12 (40%), *Streptococcus faecalis*, 8 (26.7%),

Escherichia coli, 5 (16.7%), *Klebsiella species*, 3 (10%), and *Pseudomonas aeruginosa*, 2 (6.7%).

Fig 2: Frequency of the bacterial isolates from the 32 children with ASB



Urinalysis and microscopy result of children with ASB

The dipstick urinalysis showed that 26 (81.3%) of the children with ASB had an acidic urine; 4 (12.5%) had alkaline urine while 2 (6.2%) had neutral urine. Tests for nitrites and leucocytes esterase were negative in all the subjects with ASB. There was neither blood nor glucose in their urine. Two (6.2%) of the bacteriuric children had 1+ of protein while 3 (9.4%) had trace of protein. Only one child (3.1%) had protein of 2++. Pyuria (≥ 5 WBC/hpf) was seen in only one female subject (3.1%) who had 7 WBC/hpf. The majority of the children with ASB had between 1-3 WBC/hpf while the remaining subjects had no white cells at all. There was no cast in all the cases but uric acid crystal was demonstrated in 2 (6.25%) subjects and calcium oxalate crystal in another 2 subject (6.25%).

Discussion

In this cross-sectional survey, we determined the prevalence of ASB in pre-school children in Nnewi, South-East Nigeria. The prevalence of ASB was found to be 4%. The prevalence of ASB in this study agrees with previous reports of about 3-6% in studies carried out both outside Nigeria and within the country.¹⁰⁻¹² In Europe, Nebigel *et al*¹⁰ documented a prevalence of 5.8% amongst pre-school children while Oner *et al*¹¹ reported a prevalence of 3.3%. Eyong *et al*¹² studied pre-school children in Calabar, Nigeria and found a prevalence of 5.6%. Similarly, an ASB prevalence of 7.3% was reported by Jombo *et al*¹³ in a study of pre-school children in Calabar, Nigeria.

Contrarily, some authors have reported ASB prevalence rates much higher than what we found. Kondapaneni *et al*¹⁴ reported ASB prevalence of 16.5% among 200 school children in India while Salem *et al*¹⁵ documented a rate of 30% in Egyptian children. In Nigeria, reports of higher ASB rates include 10.3% by Iduoriyekemwen *et*

*al*¹⁶ and 15% by Wogu *et al*¹⁷ both in Benin City, and 48% by Alo *et al*¹⁸ in rural primary school children in Ebonyi State.

The higher ASB prevalence in the above studies can be explained by a number of reasons. Some of the studies involved relatively lower sample sizes of 100-200.¹⁴⁻¹⁷ In addition; Iduoriyekemwen *et al*¹⁵ included a large number of infants who are known to have a higher predisposition to UTI. The fact that Salem *et al*¹⁵ studied children with type 1 diabetes and the subjects of Iduoriyekemwen *et al*¹⁶ were all HIV-infected children could have also contributed to the high values they found. While our study was carried out in the community, those of Salem *et al*¹⁵ and Iduoriyekemwen *et al*¹⁶ were hospital-based studies and as such the children are considered to have a higher risk of UTI or sample contamination. The very high ASB rate of 48% documented by Alo *et al*¹⁸ could be attributed to the fact that the study was carried out among children in a rural area who are known to have a low level of hygiene and poor health consciousness. Although most of the authors did not indicate the time between urine collection and its analysis, time lag between the two processes may also account for higher ASB rates in some of the studies due to higher false positive results. On the other hand, some other authors documented ASB prevalence rates much lower than what we found; 0.12% and 0.37% by Joseph *et al*¹⁹ in India and Yayli *et al*²⁰ in Turkey respectively. Compared to our study, Yayli *et al*²⁰ screened a much larger population of 10,289. In addition, the participants in both studies were older children of 5-14 years who are known to be less predisposed to UTI. While it can be argued that the better level of hygiene in developed countries such as Turkey may be contributory to the low ASB rates among the participants of Yayli *et al*²⁰, the same cannot be said for India which is a developing country. A number of studies in Nigeria including those involving older children have also reported lower prevalence of ASB of about 1-2%.^{7, 21}

There was no statistically significant difference in the prevalence of ASB across various ages in this study. However, the highest rate of ASB occurred amongst the 4 year olds (5.6%), followed by the 3 year olds (4.1%). The 5 year olds recorded the lowest rate of 2.0%. This is similar to the findings of Nebigil *et al*¹⁰ who recorded the highest prevalence of ASB in the pre-school age group among the 2,591 children aged 1 day to 16 years. The relatively higher prevalence of ASB in younger children can be explained by the fact that this age coincides with the period of toilet training when the child is more predisposed to urinary tract infection from faecal contamination.

In this study, the prevalence of ASB was significantly higher in females (7.2%) than in males (0.5%) giving a male to female ratio of 1:15. The female predominance was consistent across all ages. Similarly, other authors have recorded female predominance among children with ASB.^{16, 17, 22} This observation is attributed to the short female urethra, which is in close proximity to the anus from which it can be easily contaminated by faecal

matter. Conversely, some studies in Nigeria involving pre-school children reported no gender difference in the prevalence of ASB despite the slight female preponderance in their study population.^{13, 18}

In this study, *Staphylococcus aureus* (40.6%) was found to be the most predominant bacterial isolate, followed by *Streptococcus faecalis* (28.1%), *Escherichia coli* (15.6%), and *Klebsiella spp* (9.4%). *Staphylococcus aureus* has been reported as the predominant organism among children with ASB by some authors including Frank-Peterside²³ in Port Harcourt and Alo *et al*¹⁸ in Ebonyi State, Nigeria where it was isolated in 30% and 43.6% of cases respectively. The Predominance of *S. aureus* among the bacterial isolates in this study is in contrast with most reports from outside and within Nigeria where gram negative organisms dominated with *Escherichia coli* being the most prevalent organisms.^{11, 12, 16, 22}

Decline in the predominance of *Escherichia coli* as the causative agent of UTI has been attributed to improvement in the use of culture media such as MacConkey.²⁴ This culture medium acts as a selective and differential medium for gram negative bacilli. This greatly enhances the separation of groups of organisms into their respective genera and their appropriate species. MacConkey and nutrient agar media were employed in this study and they would have enhanced better differentiation and identification of the organisms isolated. Furthermore, decline in *E. coli* predominance with a shift to *S. aureus* has been attributed to the indiscriminate use and misuse of antibiotics and emergence of resistant organisms.²⁵

Significant pyuria defined as the presence of at least 5 WBC/hpf of centrifuged urine in this study was found in one bacteriuric female child. This suggests the unreliability of pyuria alone as an indicator of ASB. In agreement with our observation, some authors^{1, 26} share the same opinion that pyuria is a poor indicator of bacteriuria, and that the absence of pus cells does not rule out significant bacteriuria. Dogunro²⁶ in Port Harcourt noted that only 1.7% of school children aged 4-15 years had both significant bacteriuria and significant pyuria.

The clinical importance of ASB in children goes beyond its prevalence. It is its predisposition to renal scarring that makes it a critical issue. In one of the studies where ASB prevalence was found to be low, detailed investigations showed that a reasonable proportion of affected children had evidence of renal damage.¹⁹ In developing countries with poor hygienic conditions, ASB remains a big challenge amongst pre-school children. Incidentally, diagnostic facilities are inadequate in these settings and children with ASB who later develop renal damage may not be identified early enough for appropriate interventions aimed at halting or reversing renal damage. This means that health education on improved hygiene and screening for ASB in young children remains the economically viable option.

Our study had some limitations. We were unable to carry out radiological investigations for children with

significant bacteriuria. This would have helped in determining the presence and extent of renal pathology as well as the co-existence of congenital or structural urogenital abnormalities in these children.

unwanted consequences of bacteriuria and its higher prevalence in females, all female pre-school children should be routinely evaluated for bacteriuria as part of the school health programme.

Conflict of Interest: None.

Funding: None.

Conclusion

In conclusion, ASB remains an important problem in pre-school children. The prevalence of ASB was significantly higher in females, being 15 times that of their male counterparts. Gram positive organisms were the predominant isolates among children with ASB with *S. aureus* and *S. faecalis* accounting for over 68% of the cases. *Escherichia coli* which has been widely reported to be the predominant isolate in many studies was responsible for only 15.6% of the cases. In view of the

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