

**Original Article****Open Access****Biochemical and bacteriological profiles of asymptomatic bacteriuria among school children in Ago-Iwoye, Nigeria**

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\*Correspondence to: [benthoa2013@gmail.com](mailto:benthoa2013@gmail.com); ORCID: <http://orcid.org/0000-0003-0675-5749>**Abstract:**

**Background:** Asymptomatic bacteriuria (ASB) in children is a predisposing factor to symptomatic urinary tract infection (UTI) that may be complicated by blood stream infections if not appropriately treated with resultant mortality or morbidity. The objectives of this study are to determine the prevalence of ASB, and evaluate both biochemical and bacteriological characteristics of urine samples of primary school pupils in Ago-Iwoye, Ijebu North Local Government Area (LGA), Ogun State, Nigeria.

**Methodology:** Three hundred and seventy-two (186 males and 186 females) apparently healthy (asymptomatic) pupils aged 2-16 years from four randomly selected primary schools in the LGA were screened for ASB. Clean catch specimen of midstream urine was collected from each subject. Biochemical analysis of the urine was performed with Combi 10 reagent strip. MacConkey and Cysteine Lactose Electrolyte Deficient (CLED) agar plates were inoculated with calibrated wireloop delivering 0.01 ml of urine for aerobic culture at 37°C for 24 hours. Identification of significant bacteria on culture plates was done using conventional biochemical tests.

**Results:** The frequency of clear, slightly turbid and turbid urine were 31 (8.3%), 99 (26.6%) and 56 (15.1%) respectively. All analyzed urine samples were alkaline and negative for ketone, glucose and blood, but contained protein in 230 (61.8%), bilirubin in 184 (49.5%), nitrites in 64 (17.2%) and urobilinogen in 14 (3.7%) subjects. The prevalence of significant bacteriuria was 11.8% (44 of 372) with 7.0% in males and 16.7% in females ( $p = 0.0063$ ). The frequency of bacteria isolated in descending order were *Escherichia coli* 61.4%, *Staphylococcus saprophyticus* 61.4%, *Staphylococcus aureus* 45.5%, *Bacillus subtilis* 45.5%, *Enterococcus faecalis* 43.2%, *Enterobacter* spp 36.4%, *Serratia marscencen* 31.8%, *Klebsiella pneumoniae* 22.7%, *Proteus mirabilis* 22.7% and *Pseudomonas aeruginosa* 20.5%.

**Conclusion:** This result highlights the presence of significant bacteriuria among apparently healthy pupils in the study area, with higher prevalence in the female pupils. The apparent risk of developing symptomatic UTI with the attendant complications in these pupils should spur preventive education of parents/guardians and the general populace about this entity.

**Keywords:** Asymptomatic bacteriuria, *S. saprophyticus*, morbidity, prevalence, primary school pupils

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**Profils biochimiques et bactériologiques de la bactériurie asymptomatique chez des écoliers à Ago-Iwoye, Nigéria**

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\*Correspondance à: [benthoa2013@gmail.com](mailto:benthoa2013@gmail.com); ORCID: <http://orcid.org/0000-0003-0675-5749>**Abstrait:**

**Contexte:** La bactériurie asymptomatique chez l'enfant est un facteur prédisposant à l'infection symptomatique des voies urinaires qui peut se compliquer d'infections du flux sanguin s'il n'est pas traité correctement avec la

mortalité ou la morbidité qui en résulte. Les objectifs de cette étude sont de déterminer la prévalence d'ASB et d'évaluer les caractéristiques biochimiques et bactériologiques d'échantillons d'urine d'élèves du primaire à Ago-Iwoye, région du gouvernement local d'Ijebu North, État d'Ogun, au Nigéria.

**Méthodologie:** Trois cent soixante douze (186 garçons et 186 filles) des élèves apparemment sains (asymptomatiques) âgés de 2 à 16 ans de quatre écoles primaires sélectionnées au hasard dans la LGA ont été soumis à un dépistage du PNA. Des échantillons de capture d'urine à mi-parcours ont été recueillis chez chaque sujet. L'analyse biochimique de l'urine a été réalisée avec une bandelette de réactif Combi 10. Des plaques de gélose MacConkey et Cysteine Lactose Déficiant en électrolyte (CLED) ont été inoculées avec du fil électrolytique calibré, délivrant 0,01 ml d'urine pour une culture aérobie à 37 ° C pendant 24 heures. L'identification des bactéries significatives sur des plaques de culture a été réalisée à l'aide de tests biochimiques classiques

**Résultats:** La fréquence des urines claires, légèrement troubles et troubles était respectivement de 31 (8,3%), 99 (26,6%) et 56 (15,1%). Tous les échantillons d'urine analysés étaient alcalins et négatifs pour la cétone, le glucose et le sang, mais contenaient des protéines chez 230 (61,8%), de la bilirubine chez 184 (49,5%), des nitrites chez 64 (17,2%) et de l'urobilinogène chez 14 (3,7%). La prévalence de la bactériurie significative était de 11,8% (44 sur 372) avec 7,0% chez les hommes et 16,7% chez les femmes ( $p = 0,0063$ ). La fréquence des bactéries isolées par ordre décroissant était *Escherichia coli* 61,4%, *Staphylococcus saprophyticus* 61,4%, *Staphylococcus aureus* 45,5%, *Bacillus subtilis* 45,5%, *Enterococcus faecalis* 43,2%, *Enterobacter* spp 36,4%, *Serratia marcescens* 31,8%, *Klebsiella pneumoniae*, *Proteus mirabilis* 22,7% et *Pseudomonas aeruginosa* 20,5%.

**Conclusion:** Ce résultat met en évidence la présence d'une bactériurie significative chez les élèves apparemment en bonne santé dans la zone d'étude, avec une prévalence plus élevée chez les élèves de sexe féminin. Le risque apparent de développer une infection urinaire symptomatique accompagnée des complications associées chez ces élèves devrait inciter à l'éducation préventive des parents / tuteurs et de la population en général sur cette entité.

**Mots-clés:** bactériurie asymptomatique, *S. saprophyticus*, morbidité, prévalence, élèves du primaire

## Introduction:

The prevalence of significant bacteriuria and the consequences in children especially primary school pupils have been well documented (1,2,3). Traditionally, the presence of bacteria in urine is said to be significant when the bacterial count is more than  $10^5$  colony forming units (CFU) per millilitre of urine and such condition is not uncommon among public primary school pupils in Ogun State (4), considering their low socio-economic status and prevailing poverty that prevents access to health care service and education.

The prevalence of significant bacteriuria is known to vary from place to place. In India, Kaushik and Chaudhary reported a low prevalence of 5% significant bacteriuria among primary school pupils (5) while Mzaki *et al.*, and Ocoru *et al.*, in Tanzania and Uganda respectively reported high rates of 20.3% and 26.8% among primary school children (6, 7). In Nigeria, Aiyegoro *et al.*, reported 11.9% rate among children in Ile-Ife (8) while Ogbukagu *et al.*, reported a high prevalence rate of 17.6% among children in Anambra State (9). A previous study by Ayoade *et al.*, among University students in Redemption camp in Ogun State, Nigeria, reported 25% rate among sampled subjects (10). Due to paucity of information on this entity in our environment, this study was conducted to determine the prevalence of ASB and evaluate biochemical and bacteriological characteristics

of urine samples of apparently healthy primary school pupils in Ago-Iwoye, Ijebu-North Local Government area of Ogun State, Nigeria.

## Materials and methods:

### Study setting, design and subjects

This cross-sectional study was conducted in Ago Iwoye, Ijebu North Local Government Area (LGA) of Ogun State, Nigeria from March 2012 to March 2014. A total of 372 apparently healthy primary school pupils (186 males, 186 females) aged 2 to 16 years were enrolled into the study from 4 randomly selected public primary schools in the LGA. Informed consent of the parents/guardians of each pupil and the permission of each school head teacher were obtained. The study was approved by the Ethics Committee of the Ijebu North Local Government Area Council.

Children with fever and urinary symptoms such as dysuria, passage of dark urine, frequency, suprapubic or renal pain, facial or leg swellings, and those who have received antibiotics during 48 hours prior to sample collection were excluded.

### Sample collection

A labeled, sterile wide mouth, screw cap plastic container of 20 ml capacity was given to each parent/guardian with verbal and written instructions on the procedure for collecting mid-stream urine samples from the pupils. The samples were collected and immediately transported to the Microbiology

Laboratory of the Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria for analysis.

### Macroscopy and biochemical analysis

The urine samples were examined visually for turbidity (clear, slightly turbid or turbid) and appearance (clear, pale yellow, yellowish, amber or blood stained). The biochemical analysis was carried out using Combi 10 reagent strip (BHL Pharmaceuticals, United Kingdom) according to manufacturer's instructions. The parameters examined were pH, specific gravity, ketones, protein, bilirubin, urobilinogen, glucose, nitrite, blood and leucocytes.

### Culture and isolation of bacteria

MacConkey and Cysteine Lactose Electrolyte Deficient (CLED) agar plates (Merck, Germany) were inoculated using a calibrated sterile wire loop delivering 0.01 ml of urine sample. The plates were incubated aerobically at 37°C for 24 hours. The number of colonies counted on each plate was multiplied by a factor of 100 to estimate the colony forming units (CFU) per millilitre of urine, and significant bacteriuria was taken as 10<sup>5</sup> CFU/ml.

### Identification of bacteria

Colonies from culture plates with significant bacteriuria were first Gram stained and bacteria identified to species level using conventional biochemical tests (11, 12). The Gram positive cocci were tested for catalase, coagulase, deoxyribonuclease, mannitol fermentation, aesculin hydrolysis, and sodium chloride utilization while the Gram positive bacilli were identified by spore staining, mannitol egg yolk fermentation and sugar fermentation tests. The Gram negative bacteria were biochemically identified using oxidase, indole, citrate utilization, motility, methyl red, voges proskaeur, and triple sugar iron tests scheme (11, 12).

### Antibiotic susceptibility testing

Antibiotic susceptibility testing was done by the modified Kirby Bauer disk diffusion method (13) using the following antibiotic

disks; cotrimoxazole (25µg), erythromycin (5µg), gentamicin (10µg), augmentin (30µg), streptomycin (10µg), tetracycline (10µg) and chloramphenicol (10µg) for Gram positive bacteria while augmentin (30µg), ofloxacin (5µg), gentamicin (10µg), nalixidic acid (30µg), nitrofuratoin (200µg), cotrimoxazole (25µg), amoxicillin (25µg) and tetracycline (25µg) were used for Gram negative bacteria. The diameter of zone of inhibition was interpreted as sensitive or resistance using the guidelines of CLSI (14).

### Statistical analysis

Data were analysed with GraphPad InStat (GraphPad Software, Inc., San Diego). Association between variables was established using Chisquare test, with level of significance set at  $p < 0.05$

### Results:

A high percentage of urine samples 93 (25%) were pale yellow in colour, 54 (14.5%) deep yellow, 32 (8.6%) amber and 7 (1.8%) clear, while none of the urine sample contained visible blood. In terms of consistency, 99 (26.6%) urine samples were slightly turbid, 56 (15.1%) were turbid while 31 (8.3%) were clear. The chemical analysis of the urine samples showed 230 (61.8%) were positive for protein, 184 (49.5%) for bilirubin, 64 (17.2%) for nitrite and 14 (3.7%) for urobilinogen. Leucocyte was present in 158 (42.5%) urine samples (Table 1).

Table 2 shows the prevalence of significant bacteriuria to be 11.8% (44 of 372) with 7.0% (13 of 186) in males and 16.7% (31 of 186) in females ( $p=0.0063$ ). The frequency of each bacteria species isolated from urine in descending order as presented in Table 3 are *Escherichia coli* (61.4%), *Staphylococcus saprophyticus* (61.4%), *Staphylococcus aureus* (45.5%), *Bacillus subtilis* (45.5%), *Enterococcus faecalis* (43.2%), *Enterobacter* spp (36.4%), *Serratia marscencen* (31.8%), *Klebsiella pneumoniae* (22.7%), *Proteus mirabilis* (22.7%) and *Pseudomonas aeruginosa* (20.5%).

Table 1: Macroscopic and biochemical analysis of urine samples from school children in Ago-Iwoye, Ogun State, Nigeria

Parameters	Number	Percentage
Appearance		
Clear	5	1.3
Pale yellow	93	25.0
Deep yellow	52	14.0
Amber	222	59.7
Blood stained	0	0
Consistency		
Turbid	146	39.2
Hazy	199	53.5
Clear	27	7.3
pH		
acidic	0	0
neutral	0	0
alkaline	372	100
Specific gravity		
1.000	240	64.5
1.005	60	16.1
1.010	20	5.3
1.015	24	6.4
1.020	28	7.5
Ketones		
Positive	0	0
Negative	372	100
Protein		
Present	230	61.8
Negative	142	38.2
Bilirubin		
Positive	184	49.5
Negative	188	50.5
Urobilinogen		
Positive	14	3.7
Negative	358	96.3
Glucose		
Positive	0	0
Negative	372	100
Nitrite		
Positive	64	17.2
Negative	308	82.8
Blood		
Positive	0	0
Negative	372	100
Leukocytes		
Positive	158	42.5
Negative	214	57.5

Table 2: Significant bacteriuria among primary school children in Ago Iwoye in relation to gender

Significant bacteriuria	Gender		Total (%)
	Male (%)	Female (%)	
Positive	13 (7.0)	31 (16.7)	44 (11.8)
Negative	173 (93.0)	155 (83.3)	328 (88.2)
Total	186	186	372

$\chi^2 = 7.449, p = 0.0063$

Table 3: Distribution of bacterial uropathogens among primary school pupils in Ago-Iwoye in relation to gender

Bacteria	Male	Female	Total (%)
Gram positive			
<i>Staphylococcus saprophyticus</i>	9	18	27
<i>Staphylococcus aureus</i>	7	13	20
<i>Enterococcus faecalis</i>	7	12	19
<i>Bacillus subtilis</i>	6	14	20
Gram negative			
<i>Escherichia coli</i>	9	18	27
<i>Enterobacter</i> spp	5	11	16
<i>Serratia marcescens</i>	5	9	14
<i>Klebsiella pneumoniae</i>	2	8	10
<i>Proteus mirabilis</i>	4	6	10
<i>Pseudomonas aeruginosa</i>	3	6	9
Total	57	115	172

The isolates were counted per urine sample and all urine yielded polymicrobial growth. The total number of isolates was calculated by adding up the number of time each isolate was recovered from the 44 subjects with significant bacteriuria

## Discussion:

The prevalence rate of 11.8% for significant bacteriuria in our study compares favorably with the 11.9% rate reported by Aiyegoro et al., among children in Ile-Ife (8) which is a region in the same southwestern geographical zone as our study area. In addition, the macroscopic features of urine reported in our study are similar to those reported by Dada and Aruwa in Akure, southwest Nigeria (3). The rate in our study is however less than 17.6% rate reported by Ogbukagu et al., in southeastern zone of Nigeria (9) and also less than 25% rate reported in a previous study by Ayoade et al., among university students in Ogun State (10). This is not unexpected as rates of ASB tend to vary from region to region, and are generally higher among young adults in reproductive age group like the university students in the study by Ayoade et al., (10). The significantly higher prevalence of ASB in females in our study is in line with what has been generally established. The female urinary system is in close proximity with the anus and vaginal, and coupled with the shorter female urethra, this encourages colonization and ascending infection from normal vaginal and anal flora.

The findings on chemical analysis of the urine may reveal underlying pathology when certain analytes such as glucose, ketones or blood, which are usually not present in urine of normal persons, are found. In this study however, none of the urine samples from the children contained glucose, ketone or blood. While some authors have reported presence of blood in urine (haematuria) as a common occurrence without associated disease process within the urinary tract, others have emphasized haematuria as a sign of urinary tract pathology from infections, stones or

cancer (15, 16). The presence of blood in urine samples (terminal haematuria) is however an important marker for the diagnosis of urinary schistosomiasis in an endemic region (17). The fact that none of the urine samples in our study contained blood is an indication that the studied population had no underlying pathology especially related to schistosomiasis (18, 19, 20).

The finding of *E. coli* as the most prevalent uropathogen in significant bacteriuria in our study agrees with reports of previous studies (21, 22), and the higher prevalence in females is similar to other reports (23). This observation is also in agreement with the study of Abdul et al., who reported *E. coli* (a human faecal flora that inhabits the colon as an innocuous commensal) to be the most prevalent facultative gram negative bacillus causing UTI in their study (24). The isolation of other members of the family enterobacteriaceae such as *Enterobacter* spp, *S. marcescens*, *K. pneumoniae* and *P. mirabilis* in urine in our study is also due to the fact that these organisms are frequently present in small number as normal flora of the intestine from where they can colonize anterior urethra and be present in significant number in voided urine (25). Similarly, *Ps. aeruginosa* may be present as normal flora of the intestine and anterior urethra, however it is usually a cause of hospital acquired UTI particularly in patients with immunosuppression, malignancy, cystic fibrosis, burns and traumatic wound injuries (26).

*Staphylococcus saprophyticus* was the most prevalent Gram positive uropathogen in ASB occurring with same frequency as *E. coli* in our study. This finding agrees with the study by Ranjbar et al., (23) who reported *S. saprophyticus* as the most prevalent, but differs from those of Forouzan et al., who reported *S. aureus* as the most prevalent Gram

positive uropathogen in their study (27). *S. saprophyticus* has long been recovered as normal flora of urogenital tract of women (28) but it has also been described as the second most common cause of uncomplicated acute UTI after *E. coli* in young sexually active women in the western world (29). The high frequency of *S. saprophyticus* isolation in both male and female pupils with ASB in this study implies that this Gram positive pathogen with others such as *S. aureus* and *Enterococcus faecalis*, should be considered as significant pathogens when investigating bacteria agents of UTI in our region.

## Conclusion:

Results of the study highlights the presence of significant bacteriuria, predominantly caused by *E. coli* and *S. saprophyticus*, among apparently healthy pupils in the study area, with higher prevalence in the female pupils. The apparent risk of developing symptomatic UTI with its attendant complications in these pupils should spur preventive education of the parents/guardians and the general populace of the possible menace of this entity.

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