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URINARY TRACT MICROBIAL PRESENCE IN SCHOOL-AGED CHILDREN WITHIN A SOUTHWESTERN NIGERIAN DISTRICT

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SUMMARY – This community-based investigation evaluated how often microorganisms appear in urine among school-age populations in a defined region, and examined observed trends in relation to age and sex. The research was conducted in a school setting using volunteers across a range of grade levels. Clean catch urine samples were collected under sterile conditions and examined using multiple culture media to identify any bacteria present.

Findings suggested a higher detection rate in female participants compared to males, as well as a slightly elevated occurrence in older students. The most frequent type of bacterium detected was one commonly linked to urinary infections. Even though none of the participants exhibited overt symptoms, laboratory testing confirmed the presence of an infection in several cases.

These insights underscore the value of routine screening in school health programs to identify and manage silent infections early, especially in regions with limited health awareness. This preventive strategy may help reduce long-term complications and support better health outcomes.

Key words: *Locality; Pupils; Bacteriuria; Urine; Comparison; Nigeria*

Introduction

Bacteriuria is the presence of bacteria in urine; it is a sign of urinary tract infection (UTI). Urinary tract infection is any infection of a part of urinary tract. In 1996, Zainal and Baba defined UTI as microbial invasion of any of the tissues, of the tract extending from renal cortex to the urethral meatus[1].

Urine is expected to yield no growth on artificial medium because urine is expected to be sterile under normal conditions of good health. The bladder and urinary tract are normally sterile, but the urethra as well as the perineum may contain a few commensals (a wide variety of gram positive and gram negative organisms), which can contaminate urine when it is being collected[2].

Bacteriuria has to be considered as an abnormal laboratory finding which should always alert physicians to the presence of UTI. It is sometimes asymptomatic and it is very important in clinical findings as it may lead to detection of important structural or neurologic lesions of the urinary tract. It is also of great value in the diagnosis and management of large numbers of individuals with symptomatic UTI and also in a large number of individuals who would ex-

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hibit considerable morbidity if left unchecked[3].

Children generally have a low rate of symptomatic UTI, except that the prevalence of bacteriuria among girls has been found to be higher compared to that in boys[4]. The explanation of Bartkowski[5] is that the nature of female urethra is of much practical significance and is one of the reasons why UTI is more common in renal damage and end stage renal failure in a significant number of female patients. It is therefore an imperative to diagnose UTI early enough and treat it appropriately so as not to result in chronically ill health and long term renal damage[6]. The incidence of UTI varies in early infancy and childhood, being more common in boys in the first three months of life, with reported distribution of 5:1 with male predominance[7], whereas in later childhood, the reported male to female ratio is 1:10[8,9].

Bacteriuria is regarded to be significant when the urine contains 10^5 organisms or more *per* mL (10^{-8}/mL) in pure culture. Infection of the bladder is called cystitis. It causes frequency of urination, dysuria (pain on passing urine), suprapubic pain, sometimes hematuria, and usually pyuria (increased number of pus cells in urine), pyelonephritis (infection of the kidney), loin pain, rigors, fever and often bacteremia. The risk of infection is increased when there is urine retention due to the bladder not emptying completely, or when urinary flow is obstructed due to renal stones, urinary schistosomiasis, enlarged prostate (the most common causes of recurring UTI in men), or tumor and persistent or recurrent UTI can lead to renal failure. Chesbrough[2] and Elder[9] have shown that the factors involved in causation of bacteriuria are many and diverse, and to some extent depend on sex and age.

Some of the symptoms of symptomatic bacteriuria are very severe lower abdominal pain mainly on the affected side, high fever and splitting headache; voiding urine is too frequent and difficult. Some UTI cases become chronic, with bloody pus discharge that may eventually lead to pelvic inflammatory disease[10,11].

Materials and Methods

Sample collection

Urine samples were collected from male and female pupils at different primary and secondary schools and each sample was labeled according to the number on the questionnaire distributed to study subjects. The urine collected by the pupils was midstream urine (MSU), i.e. the middle urine flow which had spent no less than two to three hours in the urethra. The pupils were instructed on how to collect a clean-catch MSU and on the need to collect the urine with as little contamination as possible. Female pupils were taught to clean the area around the urethra opening with clean water, dry the area with a dry sterilized tissue paper provided and to collect the urine with their labia held apart. Male pupils were taught to wash their hands before collecting the sample. Each pupil was given a sterile, wide-necked, leak proof universal bottle where the sample was to be collected. The samples were immediately transferred to the laboratory for examination and microbiological analysis.

Preparation of culture media

The media used were plate count agar, mannitol salt agar, cystine lactose electrolyte deficient (CLED) agar, peptone water, and chocolate agar. The manufacturers' instructions were strict1y followed on their preparation.

Total plate count

Serial dilution was carried out on each urine sample after which 0.5 mL of 10^3 dilutions was pour plated aseptically with molten plate count agar in duplicates. The plates were swirled gently for even distribution and allowed to solidify on the bench before incubation at 37 °C for 48 h in inverted position. After incubation, the resultant colonies on the seeded plates were counted and the mean taken for analysis. This was expressed as the colony forming units (cfu) of organisms *per* mL of urine (cfu/mL).

Culture of samples and bacterial identification

Then, 0.5 mL of each of diluted samples was cultured on plate count agar for total enumeration of bacterial organisms in urine samples in CLED medium. This medium isolates both gram positive and gram negative urine pathogens. The indicator in this medium is bromothymol blue and therefore lactose fermenting organisms appear yellow; also, the medium is electrolyte-deficient to prevent swarming of the *Proteus* species. Mannitol salt agar (MSA) was used to

Table 1. Total bacterial count in urine samples from primary school male pupils

Age (yrs)	Number examined	Number infected (%)	Total plate count (cfu/mL)
5-7	26	0 (0)	$10x10^3$-$70x10^3$
8-9	14	1 (7.14)	$10x10^3$-$70x10^3$
10-11	10	1 (10)	$10x10^3$-$70x10^3$
Total	50	2 (4)	

isolate staphylococci and chocolate agar was proposed to isolate the organisms that require blood to support their growth in artificial medium. The plates were incubated at 37 °C for 48 h. Bacterial colonies on the plates after incubation were purified by sub culturing on freshly prepared nutrient agar and incubated at 37 °C for 24 h. Purity was confirmed by Gram staining and pure isolates were characterized and identified based on the criteria of Chesbrough[2], Farme and Kelly[12], and Holt *et al.*[13].

Table 2. Total bacterial count in urine samples from primary school female pupils

Age (yrs)	Number examined	Number infected (%)	Total plate count (cfu/mL)
5-7	26	1 (3.85)	$3.0x10^3$-$102x10^3$
8-9	21	4 (19)	$4.8x10^3$-$186x10^3$
10-11	19	4 (21.1)	$30x10^3$-$172x10^3$
Total	60	9 (15)	

Results

Total bacterial counts in urine samples of male and female pupils from primary and secondary schools were evaluated for the prevalence of bacteriuria. Table 1 shows total bacterial count in urine samples from primary school male pupils. In the study area, a total of 50 pupils were examined for bacteriuria count and two (4.0%) were found to be infected. The highest count was observed in the 11-20 age group, with bacterial count ranging between $172x10^3$ and $204xl0^3$ cfu/mL.

The highest bacterial count in primary school female pupils was $l86x10^3$, recorded in the 8-9 age group (Table 2). The total plate count ranged from $3.0xl0^3$ to $186x10^3$.

Table 3. Total bacterial count in urine samples from secondary school male students

Age (yrs)	Number examined	Number infected (%)	Total plate count (cfu/mL)
10-11	8	2 (25)	$124x10^3$-$70x10^3$
12-14	12	0 (0)	-
15-19	15	4 (26.7)	$96x10^3$-$149x10^3$
Total	35	6 (17.1)	

In the pupils aged 15-19 years, the highest bacterial count was $149x10^3$. The pupils aged 12-14 had no growth, i.e. the infection rate in this age group was 0% (Table 3).

Table 4 shows total bacterial count in secondary school female students. A high bacterial count was observed in all age groups. The highest count was found in the l6-18 age group ($251x10^3$) and the lowest count in the 10-12 age group ($86x10^3$). Although the number of population represented here was small, the results obtained suggested that bacterial count in urine samples could have increased with age.

The age and sex distribution of UTI in primary school pupils is shown in Table 5. Out of 110 pupils who had complaints related to UTI and who were subjected to urine culture, there were 50 (45.5%) male and 60 (54.5%) female subjects. Of these, UTI was confirmed by urine culture in only 11 (10%) subjects, nine (8.18%) female and two (1.89%) male. The male to female ratio was 1:4. Table 6 shows that the prevalence of UTI in secondary school pupils was higher in females (93.9% of 105 examined subjects) than in males (n=6; 6% of 35 examined subjects).

Table 7 shows the prevalence of bacteria detected in urine samples from male and female school pupils. *Escherichia coli* was the predominant organism with a

Table 4. Total bacterial count in urine samples from secondary school female pupils

Age (yrs)	Number examined	Number infected (%)	Total plate count (cfu/mL)
10-12	30	26 (86.7)	$86x10^3$-$214x10^3$
13-15	35	31 (88.6)	$100x10^3$-$225x10^3$
16-18	37	33 (89.2)	$97x10^3$-$251x10^3$
19-20	3	3 (100%)	$102x10^3$-$204x10^3$
Total	105	93 (88.6)	

Table 5. Sex and age distribution of urinary tract infection in primary school pupils

Age (yrs)	Male pupils		Female pupils		Total	
	Number examined	Number infected (%)	Number examined	Number infected (%)	Number examined	Number infected (%)
5-7	26	0 (0)	20	0 (0)	46	0 (0)
8-9	14	1 (7.14)	14	4 (28.57)	28	5 (17.85)
10-11	10	1 (10)	14	2 (18.28)	25	3 (12)
12-13	0	0 (0)	12	3 (25)	12	3 (25)
Total	50	2 (4)	60	9 (15)	110	11 (10)

total count of 61 colonies in pure culture: 48.8% of female urine samples and 50% of male urine samples, followed by *Staphylococcus aureus* with 32 colonies recorded from pure culture, 25.7% of female and 25% of male samples; and *Klebsiella pneumoniae* with 12.4% of female and 16.7% of male samples; *Proteus mirabilis* with 8.8% of female samples and 0% of male samples. *Pseudomonas aeruginosa* was the least frequently detected organism, found in 4.4% of male samples and 8.3% of male samples.

Discussion

The isolation and identification of bacteria in MSU as one of the causative agents of UTI in pupils of primary and secondary schools in the Akure North Government Area led to the diagnosis of unexplained asymptomatic infection in the pupil population.

Table 6. Sex and age distribution of urinary tract infection in secondary school pupils

Age (yrs)	Male pupils		Female pupils		Total	
	Number examined	Number infected (%)	Number examined	Number infected (%)	Number examined	Number infected (%)
10-11	8	2 (25)	20	17 (85)	28	19 (67.85)
12-14	12	0 (0)	30	27 (90)	42	27 (64.28)
15-17	12	3 (25)	50	44(88)	61	47 (77.05)
18-20	4	1 (25)	5	5 (100)	9	6 (66.66)
Total	35	6 (17.14)	105	93 (88.57)	140	99 (70.71)

Table 7. Occurrence and frequency of organisms isolated as pure culture from urine samples of pupil population in Akure North Local Government Area

Organism	Male pupils	Female pupils	Total isolates	Total % of isolates
Staphylococcus aureus	3	29	32	25.6
Escherichia coli	6	55	61	48.8
Citrobacter freundii	0	0	0	0
Klebsiella pneumoniae	2	14	16	12.8
Proteus mirabilis	0	10	10	8
Pseudomonas aeruginosa	1	5	6	4.8
Total	12	113	125	100

Out of 250 specimens investigated, bacterial colonization was identified as the sole or major cause of UTI in 44% of samples, with special emphasis and focus on the bacteria as the causative agent of UTI. In this study, *Escherichia coli* were predominant (48.8%) with a total colony count of 61 colonies in pure culture. This finding is in agreement with the reports by Zainal and Baba[1] and Bergston[14], stating that it is important to diagnose and treat UTI in children before renal damage has taken place. The latter study was aimed at assessing the efficacy of urinary nitrite in screening for symptomatic bacteriuria among school children. Out of 44,816 school children investigated,

240 (0.54%) students were judged to have bacteriuria, i.e. 82 (0.18%) boys and 158 (0.35%) girls, where *Escherichia coli* was the organism most commonly isolated in this study 28.75%. The second most common organism in this project work was *Staphylococcus aureus* (25.6%) with a high total colony count of 32 colonies recorded from pure culture[14]. The reason for this might be urethritis caused by infection of the anterior urinary tract or possible human contamination because it was present in 25.6% of urine specimens. About 30% of normal human beings harbor staphylococcus on their skin and this organism is a good indicator of the standard of hygiene during handling; the bacterium is widely distributed in the environment, and it is present in urine specimens from both sexes, also on dirty surfaces, tools and hands, thereby being transferred to the urine specimen on collection and investigation if proper care is not taken before, during and after sample collection, handling and investigation[15].

Chesbrough[2] reports that staphylococcus infection can be associated with hospital acquired infection, often following catheterization or gynecologic surgery. It is obvious that a considerable proportion of all these pupils are likely to contaminate it with staphylococci either directly by nasal discharge or with their hands. The danger is always increased when pupils have respiratory infections causing sneezing and coughing, also from students suffering from infected cuts or from infections such as boils and pimples in which *Staphylococcus aureus* is the common causative agent[16].

Recovery of *Klebsiella pneumoniae*, which was the third most prevalent organism in this study, accounted for 12.8%. This may be due to the health status of these pupils and is in agreement with the work by Duel[17], where he categorically states that *Klebsiella pneumoniae* is found among malnourished persons and it can also be associated with hospital acquired infection of the urinary tract. Therefore, the implication of the *Klebsiella pneumoniae* presence is likely to be that these children are malnourished and could have acquired infection at the hospital. *Proteus mirabilis* and *Pseudomonas aeruginosa* accounted for 10 (8%) and 6 (4.8%) cases detected from pure culture. Conway *et al.*[18] demonstrated *Proteus mirabilis* to be associated with renal stone, and *Pseudomonas aeruginosa* to be found in hospital environments (sinks, cleaning buckets, drains, humidifiers, etc.). Many *Pseudomonas aeruginosa* infections are opportunistic hospital-acquired infections affecting those already in poor health and immune suppressed conditions[2].

Proteus mirabilis and *Pseudomonas aeruginosa* have been implicated in aerosol and the environment used during the collection and transportation of urine specimens. Therefore, contamination of urine specimens during collection and transportation is usually more frequent in Nigeria, especially where sanitary conditions are generally poor. The method of collecting urine specimens into universal bottles by the pupils may result in sample contamination; transportation of urine specimens to the laboratory where the samples are to be examined may also contribute to their contamination with pathogenic organisms.

Comparison of the results recorded in primary and secondary school pupils showed a significantly higher total number of isolates in secondary school pupils. This may be the result of sexual interaction and other infections due to inappropriate practice of hygiene like sharing underwear with other people, exposure of their private parts to public toilet, bathroom and method of cleaning up after using toilet, dysfunctional voiding and other unhygienic practice in this age group. This has already been explained by Duel[17]: when a female child does not wipe correctly or thoroughly, bacteria from the rectum can enter the urethra and result in UTI. Mohanna and Raja'a[19] also explain that when children do not empty their bladder completely, bacteria will build up. Of the cultured urine samples, 68 (27.2%) out of 85 (34%) samples examined yielded no growth. However, these were the samples collected from male pupils in secondary schools. The reason may be that most of these pupils were circumcised or they collected urine specimen properly according to the instructions given to them. Likewise, a high number of contaminated specimens were recorded in primary school pupils (44.4% of the total number examined). The reason may be that these children were unable to collect the specimens correctly according to the instructions given to them.

Considering this high count in secondary school pupils, it was observed that many samples from male pupils showed no bacterial growth. This is an impression that boys have a low rate of bacteriuria. The reason may be that they were all circumcised or they collected the urine specimens properly according to the

prescribed instruction.

This is also in agreement with Wiswell[20], stating that recurrent UTI occur in 19% of uncircumcised boys, but none of the circumcised, suggesting that circumcision reduces UTI incidence by 80 percent. Few male pupils show high counts, which could be due to improper care of the intact penis[8].

Although a high number of contaminated specimens were recorded in primary school children, this may be the result of induced contamination as these children were unable to collect the specimens correctly according to the given instructions due to their younger age, since MSU was used in this study[2].

The presence of mixed cultures, which occurred in few specimens of the pupils' population may indicate contamination. Contaminating organisms usually produce a few colonies of mixed growth of a single type of organism; however, mixed growth can also occur, especially in chronic infection[1]. This finding is also in agreement with the report of Chesbrough[2], where it is categorically stated that most UTI show growth of a single type of organism but mixed growth can occur especially in chronic infections or after catheterization or gynecologic surgery.

The studies by Larcombe[21], Mehr *et al.*[22], Elegbe *et al.*[23] and Saleh *et al.*[4] have revealed that *Escherichia coli* still accounts for a greater percentage of UTI (48.8%). This organism is the commonest urinary pathogen causing 60% to 90% of infection[6]. Also, Conway *et al.*[18] report that *Escherichia coli* is common in patients with cystitis and recurring infections mainly in girls.

The presence of other gram negative bacilli that show tremendous increase in number might be associated with the improvement in the use of isolating media such as the CLED medium, which acts as a selective and differential medium for organisms such as *Escherichia coli, Klebsiella pneumoniae* and other non-lactose fomenters.

Our study results were also consistent with the report of Saleh *et al.*[4], proving that the rate of symptomatic UTI in children is low; most of the pupils with bacteriuria had no sign of infection (90%) and the infection was more prevalent in girls than boys; out of 125 specimens investigated, 113 (45.2%) were female and 12 (4.8%) ma1e. The reason for this high prevalence rate among girls might be the nature of female urethra, which is of much practical significance and is one of the reasons why tract infections are more common in this group[24].

In this study, bacteriuria cases were judged according to the explanation given by Paterson[3], that a bacterial count greater than one hundred multiplied by the dilution factor of 10^3 colonies cell *per* mL or more from a fresh 'clean-catch' urine specimen indicates urinary infection, whereas a count of less than 100 colonies cells *per* mL (cell/mL) is nearly always due to contamination unless the urine was cultured after antimicrobial treatment had been started. Therefore, it is necessary to estimate the approximate number of bacteria in urine because normal urine specimens may contain a small number of contaminating organisms, usually less than 10,000 (10^4) *per* mL of urine. Urine from a person with an untreated acute UTI usually contains 100,000 (10^5) or more bacteria *per* mL[2].

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