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COMPARATIVE STUDIES OF ASYMPTOMATIC BACTERIURIA AMONG SECONDARY SCHOOL STUDENTS IN RURAL AND URBAN AREAS OF OWERRI MUNICIPAL, NIGERIA

Nwachukwu, I. O.
Department of Microbiology, Imo State University, Owerri, Imo State
Email: ogechinwachukwu2081@gmail.com

ABSTRACT

Asymptomatic bacteriuria (AB) is common, with varying prevalence by age and gender amongst other factors. AB is more likely to develop to symptomatic urinary tract infection, thus, necessitating periodic review. This study aimed to determine the distribution of AB among secondary school students in rural and urban areas of Owerri municipal, Nigeria. Clean-catch mid-stream urine was obtained from 240 apparently healthy secondary school students in Owerri municipal. The subjects consists of 120 (60 males and 60 females) Junior Secondary School (JSS) students from both rural and urban areas and 120 (60 males and 60 females) Senior Secondary School (SSS) students from both rural and urban areas. The urine specimens were processed to diagnose AB. Significant emergent colonies were identified. The distribution of AB was significantly higher (P = 0.039) among SSS students (15.00%) compared to JSS students (6.67%). Generally as well as among JSS and SSS students, the distribution of AB did not differ significantly by gender (P > 0.05). *Staphylococcus aureus* was the predominant isolate causing AB among the students [4 (15.38) versus 5 (19.23)] in the urban and rural areas respectively. *S. aureus* was more common among the JSS students. In terms of gender, *S. aureus* occurs most in males in both rural 4 (15.38%) and urban 3 (11.54%) areas. Good personal hygiene and routine screening program at school age is thereby recommended.

***Keywords*:** asymptomatic bacteriuria, urinary tract infection, *S. aureus*, significant, students

INTRODUCTION

Bacteriuria is the presence of bacteria in urine [1]. Bacteriuria accompanied by symptoms is urinary tract infection while that without symptoms is known as asymptomatic bacteriuria [2]. Diagnosis is by urinalysis or urine culture. *Escherichia coli* is the most common bacterium found. People without symptoms should generally not be tested for the condition. If symptoms are present treatment is generally with antibiotics. Bacteriuria without symptoms generally does not require treatment [3]. Exceptions may include pregnant women, those who have had a recent kidney transplant, young children with significant vesicoureteral reflux and those undergoing surgery of the urinary tract.

Bacteriuria without symptoms is present in about 3% of otherwise healthy middle aged women [4]. In nursing homes, rates are as high as 50% among women and 40% in men. In those with a long term indwelling urinary

catheter rates are 100%. Up to 10% of women have urinary tract infection in a given year and half of all women have at least one infection at some point in their lives [5], [6].

Asymptomatic bacteriuria (AB), the presence of bacteria in the urine without accompanying clinical symptoms of urinary tract infection (UTI), has garnered increasing attention due to its potential long-term consequences, particularly among adolescents. The adolescent age group, undergoing critical physical and hormonal changes, represents a unique demographic where the distribution and impact of AB may differ [7].

Understanding the distribution of AB among secondary school students is pivotal as this age group is particularly vulnerable to the implications of urinary tract infections. While AB is generally considered benign, it can serve as a precursor to symptomatic UTIs, renal complications, and other infectious diseases. The choice to focus on both rural and urban settings stems from the recognition that environmental, socio-economic, and healthcare access factors may significantly influence the distribution of AB in these distinct contexts [8].

The existing literature on AB has primarily centered on adult populations, leaving a notable gap in our understanding of how this condition manifests in adolescents, especially in different geographical settings. Prior studies have identified socio-economic factors, access to healthcare, and environmental conditions as influential elements in the distribution of infectious diseases. However, the extent to which these factors contribute to AB in secondary school students in rural and urban areas remains largely unexplored.

Several studies have underscored the importance of age-specific research, given the unique physiological and behavioral characteristics of adolescents. Hormonal changes, sexual activity, and hygiene practices during this period may all contribute to the susceptibility of this demographic to urinary tract infections, making it imperative to explore the distribution of AB and its associated risk factors comprehensively among secondary school students in both rural and urban areas of Owerri municipal.

MATERIALS AND METHODS

Specimen Collection

Two hundred and forty (240) mid-stream urine specimens were collected with sterile specimen containers randomly from 240 secondary school students within the age group of 10-18 years in both rural and urban areas of Owerri municipal, Imo State, Nigeria. The urine specimens were collected after the urethra opening of the students have been cleansed with a sterile pad to avoid contamination of the specimens and the specimens were taken to the Microbiology Laboratory within 30 minutes after collection to avoid sedimentation.

Bacterial Analysis of Specimens

A loopful specimen of freshly collected urine in sterile specimen containers were inoculated on nutrient agar (for total heterotrophic plate count), MacConkey agar (for coliform count), mannitol salt agar (for total staphylococcal count) and cysteine lactose electrolyte deficient (CLED) agar using the streak plate method. The media

were incubated at 37°C for 24hrs. After the incubation period, the plates were observed and colonies counted and estimations of total bacteria made from it.

The colonies were purified by sub culturing them onto fresh nutrient agar plates and incubated at 37°C for 24hrs. The pure culture growth was used for gram staining, motility test and biochemical characterization of the bacterial isolates.

Identification of Bacterial Isolates

The bacterial isolates were identified using colonial morphology, Gram staining, motility test and biochemical properties. Biochemical test includes oxidase test, citrate utilization test, indole test, voges-proskauer test, methyl-red test, coagulase test, sugar fermentation test and catalase test

Colonial and cellular characteristics

Colonial and cellular characteristics were used in the identification of microbial isolates and they include the shape, colour, elevation, pigmentation, consistency, surface appearances and size of the colonies [9].

Gram staining

Gram staining method described by [10] was adopted. Smears of the isolates were made on clean grease-free glass slides with the aid of a sterile inoculating wire loop, air-dried and heat-fixed over a Bunsen flame. Afterwards, each smear was covered with a crystal violet stain for 30 seconds and washed off with clean water. The smear was flooded with Lugol's iodine for 60 seconds and then decolorized with 75% alcohol for 30 seconds. It was washed off quickly with clean water and counter stained with safranine for 30 seconds. The safranine stain was washed off quickly with clean water. The slides were allowed to air-dry. The smear was then examined microscopically using the oil immersion objective (X100).

Motility test

The method described by [9] was adopted. This test was carried out using the stab method. Test tubes of semi-solid motility medium was inoculated by stabbing a sterile straight wire loop charged with inoculums from the isolated pure culture vertically into the media and it was incubated at 37°C for 24hours. Non-motile bacteria produced growths that were un-diffused from the line of stab while motile bacteria produced diffused growth away from the line of stab into the medium and rendered it opaque.

Biochemical tests

The following biochemical tests were used in the identification of bacteria.

Catalase test

The method as described by [11] was adopted. This test is used to differentiate catalase producing bacteria like *Staphylococci* from non catalase producing bacteria such as *Streptococci*. A drop of 3% hydrogen peroxide was placed on each end of a microscope slide with the aid of a sterile wire loop, colonies of the test organisms was transferred on to one end of the microscope slide, and the other end was not inoculated but served as a control. The presence of gas bubbles indicates a positive catalase test

while absence of bubbles indicates a negative catalase test.

Citrate utilization test

This test is used to identify members of the family *Enterobacteriaceae*. The method as described by [9] was adopted. The test was carried out by inoculating sterilized Simmon's citrate agar with the test organisms using a sterile wire loop, incubating at 37°C for 48hrs and observing for changes in colour. Positive result shows a change of the medium colour from green colour of to royal blue colour, indicating the presence of citrate utilizing bacteria.

Coagulase test

It is used for the identification of *Staphylococcus aureus*. The method as described by [9] was adopted. A drop of distilled water was placed on each end of the microscope slide. A colony of test organism was emulsified in each of the drops of distilled water that was placed on the ends of the microscopic slide to make thick suspensions. A 100cfu/l of plasma was added to one of the suspension and mixed gently. No plasma was added to the same suspension serving as control. Clumping of the mixture within 10secs indicates positive coagulase test while absence of clumps within 10secs indicates a negative result.

Indole test

The method of [9] was adopted. Testing for indole production is important in the identification of enterobacteria. The test organisms were inoculated in bijou bottles containing 3ml of sterile tryptone water which were incubated at 37°C for 48hrs. After incubation, 0.5ml of Kovac's reagent was added. The tubes were gently shaken and the appearance of a red surface layer within 10mins indicates a positive indole test.

Oxidase test

The method as described by [9] was adopted. The test is used to assist in the identification of *Pseudomonas, Neisseria, Vibrio, Brucella, Pasturella* species, all of which produce the enzyme cytochrome oxidase. A piece of filter paper was soaked with a few drops of oxidase reagent (tetra-ethyl-p-phenylendeaminedihydrochloride). A colony of the test organism was picked with a sterile glass rod and smeared on the filter paper. A blue purple colour develops within a few seconds if the organism is an oxidase producer as a result of the oxidation of the phenylendeamine while the absence of a blue purple colour indicates a negative result.

Methyl red/ voges-proskauer (MR/VP)

This test is used to differentiate bacteria that are capable of fermenting glucose with the production of enough acid to lower the pH of the medium to 4 - 4.5 and that ferment glucose without much acid production. The method as described by [9] was adopted. The bacteria isolate was inoculated into 2ml of glucose phosphate (peptone water) and was incubated at 37°C for 48hrs. After incubation, 4 drops of methyl red indicator was added to the tube. The solution was homogenised and observed immediately for colour change. The appearance of a red colour indicates a positive result while the appearance of a yellow colour indicates a negative result. For

Voges-proskauer test, the bacteria isolate was added to 2ml of glucose phosphate (peptone water) and it was incubated at 37°C for 48hrs. After incubation, 40% potassium hydroxide and 3ml of 5% alcoholic alpha-naphthol was added. The appearance of a pink colour after 2-5 minutes indicates a positive result.

Sugar fermentation test

This test was employed to check for the ability of bacteria isolate to ferment sugar-glucose, lactose and sucrose, produce gas, hydrogen sulphide. The test bacteria were inoculated on triple sugar iron slanted. A colour change from pink to yellow indicates the utilization of several sugars. A black duct at the slanted area indicates the presences of H_2S. A gaseous bubble at the bottom of the slant indicates the presence of gases while displacement in the Durham's tube indicates gas production.

Statistical Analysis

The data obtained was subjected to analysis of variance (AVOVA) test to determine the significant difference at 95% confidence limit.

RESULTS

Table 1 shows the average bacterial load from secondary school students in urban areas. The total heterophilic bacteria, coliform and staphylococcal counts are 3.1 x 10^5 cfu/ml, 2.2 x 10^5 cfu/ml and 2.0 x 10^5 cfu/ml respectively.

Table 1. Average Bacterial Load from secondary school students in urban area of Owerri municipal, Imo State

Average Total Viable Counts (x 10^5cfu/ml)		
THBC	TCC	TSC
3.1	2.2	2.0

Keys: THBC = Total Heterophilic Bacteria Count
TCC = Total Coliform Count
TSC = Total Staphylococcal Count

Table 2 shows the average bacterial load from secondary school students in rural areas with significant microbial growth than secondary school students in urban areas.

Table 2. Average Bacterial Load from secondary school students in rural area of Owerri municipal, Imo State

Average Total Viable Counts (x 10^5Cfu/ml)		
THBC	TCC	TSC
3.6	2.9	2.4

Keys: THBC = Total Heterophilic Bacteria Count
TCC = Total Coliform Count
TSC = Total Staphylococcal Count

The distribution of asymptomatic bacteriuria (Table 3) was significantly higher (P = 0.039) among SSS than JSS students (15.00% versus 6.67%, respectively).

Table 3. The Distribution of Asymptomatic Bacteriuria among Secondary School Students in rural and urban areas of Owerri municipal, Imo State

School	No. tested	No. infected (%)	Rural (%)	Urban (%)
JSS	120	8 (6.67)	5(4.17)	3(2.50)
SSS	120	18 (15.00)	10(8.33)	8(6.67)

In both JSS and SSS students, the distribution of AB did not differ significantly (P = 0.05) between males and females (Table 4).

Table 4. Gender distribution of Asymptomatic Bacteriuria among Secondary School Students in rural and urban areas of Owerri municipal, Imo State

School/gender	No. tested	No. infected (%)	Rural(%)	Urban(%)
Junior Secondary School				
Male	60	4 (3.33)	2 (1.67)	2 (1.67)
Female	60	4 (3.33)	3(2.50)	1 (0.83)
Senior Secondary School				
Male	60	8 (6.67)	3 (2.50)	3 (2.50)
Female	60	10 (8.33)	7 (5.83)	5 (4.17)

Staphylococcus aureus was the most predominant etiologic agent of AB with a percentage frequency of 34.61% while *Klebsiella* species (3.85%) was the least cause of AB (Table 5). *S. aureus* was the predominant bacterial isolate in both the urban and rural areas of Owerri municipal, Imo State whereas *E. coli* occurs mostly in the rural areas.

Table 5. Distribution of uropathogens among secondary school students in urban and rural areas of Owerri municipal, Imo State

Organisms	Urban (%)	Rural (%)	Total (%)
Escherichia coli	2 (7.69)	4 (15.39)	6 (24.08)
Klebsiella species	0 (0.00)	1 (3.85)	1 (3.85)
Staphylococcus aureus	4 (15.38)	5 (19.23)	9 (34.61)
Coagulase negative	3 (11.54)	2 (7.69)	5 (19.23)

Staphylococcus			
Proteus species	2 (7.69)	3 (11.54)	5 (19.23)
Total	11(42.31)	15(57.69)	26 (100)

Table 6 shows the gender distribution of uropathogens among secondary school students in urban and rural areas of Owerri municipal, Imo State. *S. aureus* occurs mostly among the males while *E. coli* occurs mostly among the females.

Table 6: Gender distribution of uropathogens among secondary school students in urban and rural areas of Owerri municipal, Imo State

Organism	Urban		Rural	
	Male (%)	Female (%)	Male (%)	Female (%)
Escherichia coli	0 (0.00)	2 (7.69)	1 (3.85)	3 (11.54)
Klebsiella species	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.85)
Staphylococcus aureus	3 (11.54)	1 (3.85)	4 (15.38)	1 (3.85))
Coagulase negative *Staphylococcus*	2 (7.69)	1 (3.85)	1 (3.85)	1 (3.85)
Proteus species	1(3.85)	1 (3.85)	1 (3.85)	2 (7.69)

DISCUSSION

Asymptomatic bacteriuria (AB) in children is a significant source of morbidity and can predispose to recurrent UTI (Moses *et. al*., 2012). The distribution of AB is influenced by age, gender, sexual activity, amongst other factors [4]. This study focused on the distribution of AB among secondary school students in urban and rural areas of Owerri municipal, Imo State, Nigeria. A total of 26 (10.83%) of the 240 student had AB. This was similar with the 10% previously reported by [12]. The finding that the occurrence of AB was significantly higher in SSS students compared with JSS students (P = 0.039) agrees with the notion that UTI increases with age [4]. Sexual intercourse, a known risk factor for UTI, may be more common in SSS students. Females are known to have higher

prevalence of UTI including AB, due to the close proximity of female urethral meatus to the anus, shorter urethra and sexual intercourse [13]. A number of authors have reported higher occurrence of AB in females [12], [13]. However, it was not statistically significant.

In this study, *S. aureus* (34.61%) was the most predominant aetiologic agent of AB generally among the students. This is in agreement with a previous report by [12]. Several reports from Benin City, Nigeria, among different populations, had revealed *S. aureus* as the predominant agent of AB [13], [14]. This may explain the findings of this study. *E. coli* predominated among SSS students. It is important to note that the study of [12] did not differentiate their students into JSS and SSS, and that the change in aetiologic agents of AB between JSS and SSS students may indicate change in AB pathogen with age.

In relation to gender and with the exception of female SSS students where *E. coli* was the most predominant, *S. aureus* was the predominant organism in both genders. *S. aureus* is a normal flora of the female perineum and vulva, and can easily be carried into the urethra during sexual intercourse by a massaging process [15].

CONCLUSION

In conclusion, the presence of asymptomatic bacteriuria was noticed amongst secondary school students in both rural and urban areas of Imo State. *E. coli*, *S. aureus*, coagulase negative *S. aureus*, *Klebsiella* and *Proteus* species were isolated from the students. *S. aureus* was implicated as the commonest causative agent. Good personal hygiene and routine screening program at school age is thereby recommended. Education on abuse and unrestricted use of antibiotics should be encouraged. Further studies should be undertaken to determine the risk factors and possible sensitivity pattern among the age group.

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