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ASYMPTOMATIC BACTERIURIA AMONG FEMALE STUDENTS OF A TERTIARY INSTITUTION IN SOUTHEAST NIGERIA

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ABSTRACT

Urinary tract infections (UTI) are among the most common bacterial infections in humans. Asymptomatic bacteriuria has been observed to be a strong criterion for urinary tract infections; it is therefore a means of predicting UTI. In this study, we investigated the prevalence of asymptomatic bacteriuria among female students of Imo State University Owerri (IMSU), a tertiary institution in southeast Nigeria. Mid stream urine specimens were collected from 253 apparently healthy asymptomatic undergraduate female students of IMSU between ages of 17 and 26 who were randomly sampled. Each specimen was cultured on CLED and MacConkey agar, incubated overnight at 37^0C. Bacteria isolates were characterized and identified based on conventional microbiological techniques. Antibiotic susceptibility tests were performed using Kirby-Bauer's disc diffusion method according to performance standards of Clinical Laboratory Standard Institute guidelines. The results showed that, the total prevalence rate of bacteriuria was 13.8% with the highest rate occurring in students within age group of 20-22 (8.3%). Out of the 253 urine specimens cultured, a total of 35 bacteria isolates were identified comprising *Escherichia coli* (37.9%), *Staphylococcus aureus* (20.7%), *Klebsiella pneumonia* (17.2%), *Pseudomonas aeruginosa* (13.8%) and *Proteus mirabilis (*10.3%). All isolates tested were 100% susceptible to ciprofloxacin, ofloxacin and gentamicin but showed 100% resistance to amoxycillin and cotrimaoxazole. *Proteus mirabilis* were 100% sensitive to all antibiotics except cotrimaoxazole and amoxycillin. *Staphylococcus aureus* and *Escherichia coli* exhibited multidrug resistance to antibiotics belonging to ≥ 4 antibiotic classes. *Escherichia coli* were the most common bacteria for asymptomatic bacteriuria in female university undergraduates in this study. Statistical analysis showed that bacteriuria rate among the students residing in different hostels was not significantly different in any of the sampled hostels $P<0.05$. The incidence of the bacteriuria in a healthy population is of significant public health concern, thus, education and awareness on the need for proper personal hygiene should be encouraged.

KEYWORDS

Bacteriuria, Antibiotic resistance and Female Students.

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INTRODUCTION

Asymptomatic bacteriuria is defined as significant bacterial count in the urine, (usually ≥ 10^5 CFU/ml) of an individual without symptoms of urinary tract infections (UTI) (Frank-Peterside and Wokoma, 2009; Smith, 1994). Stamm and Hooton, 1993 referred UTI as a clinical (symptomatic) or subclinical (asymptomatic) disease that may involve just the lower tract or both the lower and upper urinary tracts. Urinary tract infections are "uncomplicated" when

they occur in a normal urinary tract with no structural, functional or underlying host illness to account for the infection, or "complicated" when an underlying abnormality is thought to have enabled the infection to occur (Krieger, 2002). Infection may involve single sites such as urethra - urethritis, prostrate-prostitis, bladder, cystitis, and kidney - pyelonephritis, however, the whole system is always at a risk of invasion by bacteria once any part is infected. Moreover, pyuria as evidenced by the inflammation of the genitourinary tract is common in subjects with asymptomatic bacteriuria (Nicolle *et al.,* 2005).

Urinary tract infections represent one of the most common diseases encountered in medical practice today and occurring from the neonate to the geriatric age group (Kunin, 1994). Despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population (Tambekar *et al.,* 2006). It occur as a result of the microbial colonization of urine and the invasion of any structure of the urinary tract by microbial organisms such as bacteria, viruses, yeasts and protozoa (Wyngaarden *et al.,* 1992). Bacterial organisms typically reported to be responsible for urinary tract infections include *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Staphylococcus saprophyticus, Enterobacter* spp., and *Proteus* spp. Worldwide, *Escherichia coli* cause 75%–90% of acute uncomplicated cystitis while *Staphylococcus saprophyticus* accounts for 5% to 15%, mainly in younger women (Gupta *et al.,* 2001; Ronald, 2002; Fihn, 2003; Mwaka *et al.,* 2011). However, the prevalence and degree of occurrence of one or two of these organisms over the other are dependent on the environment (Kolawole *et al,* 2009; Ayoade *et al.,* 2013).

Asymptomatic bacteriuria occurs when urinary tract pathogens enter into the bladder without causing apparent symptoms. Typically the pathogens are usually eliminated by host defense factors when they persist only for a short time in the human host, however when such pathogens stay in the urinary system for a long time, symptomatic urinary tract infections result (Ayoade *et al.,* 2013).

The rate of bacterial resistance to antimicrobial agents is on the increase in many countries (Ho., *et al.,* 2004). For the past two decades, trimethoprim-sulfametroxazole (SXT) or trimethoprim alone have been used widely as empirical therapy for *E.coli* UTI. However, in the United States, resistance to SXT among *E. coli* isolates from persons with UTIs has Increased substantially over the past decades, with a prevalence exceeding 20% in many parts of the country (Stamm, 2001). In Nigeria, the rate of bacterial resistance to antimicrobial agents in the management of UTIs is particularly high due to high prevalence of fake and substandard drugs (Raufu, 2002; Mordy and Erah, 2006). Asymptomatic parasitic infections are endemic in Nigeria and are usually neglected until infections become symptomatic with adverse effects particularly in immunocompromised individuals infected with secondary urinary tract pathogens. Therefore, it is necessary to regularly investigate the incidence of asymptomatic bacteriuria in apparently healthy population and also to study the antibiotic susceptibility pattern of the bacteria isolated; which will provide information on the likely choice of antibiotics to treat infections that might arise from these organisms. The present study therefore, seeks to determine the prevalence rate of asymptomatic bacteriuria in Owerri southeast Nigeria, using the female students of Imo State University as a case study. The susceptibility of the isolated bacteria to commonly used antibiotics in the region was also investigated and our result is expected to provide useful information to assist physicians and other health care providers towards developing a better management strategies of urinary tract infections.

MATERIAL AND METHOD

Study Population and Sample Collection

The study population comprised of 253 female undergraduate students of Imo State University Owerri residing in different private hostels within Owerri metropolis. Those recruited for this randomized cross-sectional study were volunteers, who gave their informed consent, non-pregnant students, no complain of symptoms of urinary tract infections and those who were not on antibiotic therapy at the time of sample

collection, or who had not taken antibiotics within one month prior to sampling. The volunteered students were taught how to collect mid-stream urine specimen and each student was then given two sterile, wide-mouthed and capped sample bottles for sample collection. Specimens of early morning, mid-stream, and clean-catch urine were collected in 20-mL volumes, properly labeled and immediately conveyed to the Laboratory for analysis. The specimens were analyzed within 4 hours of collection to ensure integrity of samples, accurate identification of pathogens and also to avoid possible proliferation of pathogens and contaminations. All sampling procedures were in accordance with guidelines of the National Health Research Ethics Committee, Nigeria (www.nhrec.net).

Isolation, Identification and Enumeration of Bacteria.

A loopful (0.002 ml) of well mixed urine was taken from each specimen using a standard sterile wire loop and inoculated on a Cysteine Lactose Electrolyte-Deficient (CLED) and MacConkey agar (Oxoid, England). The cultured plates were incubated aerobically overnight at 37˚C. Culture plates without visible growth were further incubated for an additional 24 hours before being discarded. The number and types of colonies grown on the medium (CLED) was recorded as being insignificant when samples gave a colony count of $\leq 10^4$ CFU/ml. Samples with colony count $\geq 10^5$ CFU/ml of the urine specimens were considered to have significant bacteriuria (Nicolle *et al.*, 2005). Bacterial isolates were identified based on a combination of cultural, morphological and biochemical characteristics (Cheesbrough, 2007).

Antimicrobial Susceptibility Testing

Susceptibility of the isolates to antibiotics was tested using modified Kirby– Bauer disc diffusion method on Mueller Hinton agar (Oxoid, England) against the following eight antibiotics: Amoxycillin (10 µg), Erythromycin (10 µg), Tetracycline (30 µg), Gentamicin (10 µg), Cotrimaoxazole (25 µg), Chloramphenicol, Ofloxacin(30 µg), Nalidixic acid (30 µg), Ciprofloxacin, and Cefuroxime (30 µg). (Oxoid, England). The results of the antibiotics susceptibility were interpreted based on the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2008). 0.5 Mac Farland standard was used to standardize the test inoculums.

Statistical analysis

Comparative bacteriuria rate among the students from different hostels were statistically analyzed by T-test and results were considered significant at 95% confidence level.

RESULTS

In general, the total prevalence rate of bacteriuria among the female students recruited for this study was 13.8% (35 out of 253) (single colony count $\geq 10^5$ CFU/ml). Considering the specific age groups of the students sampled; the highest prevalence rate of 8.3% (21 out of 135) was observed in students within the age group of 20-22 while the least occurrence rate of 1.2% (3 out of 21) was observed in the age group 26 and above. Most students in our study population fall within the age group 20-22 (53.4%) i.e. 135 out of 256 while the least population were in the age group 17-19, (21 out 253 i.e. 8.3%) Table No.1. A total of 35 bacteria isolates were identified from the urine specimens and these were *Escherichia coli* (37.9%), *Staphylococcus aureus* (20.7%), *Klebsiella pneumonia* (17.2%), *Pseudomonas aeruginosa* (13.8%) and *Proteus mirabilis (*10.3%). At least one isolate of *Escherichia coli and Staphylococcus aureus* were isolated from the specimens of the students in all the hostels sampled. The detailed distribution of the bacterial isolates in each of the student hostels is shown in Table No.2.

The result of the antibiotic susceptibility tests showed that all the isolates were 100% sensitivity to ciprofloxacin, ofloxacin and gentamycin but showed 100% resistance to amoxycillin and cotrimaoxazole (Table No.3). *Proteus mirabilis* were 100% sensitive to all antibiotics except cotrimaoxazole and amoxycillin. *Staphylococcus aureus* and *Escherichia coli* exhibited multidrug resistance to antibiotics belonging to ≥ 4 antibiotic classes.

DISCUSSION

Asymptomatic bacteriuria occurs frequently and is a major cause of UTI (Nurullaev, 2004). This is because

under favorable conditions, asymptomatic bacteriuria progresses to symptomatic (clinical) UTI (Harrington and Hooton 2000; Scholes *et al*., 2000). Bacteria that colonize the urinary tract may ascend towards the bladder to cause cystitis, which is usually associated with the classic symptoms of UTI (i.e., pain, frequency, and urgency). Urinary tract infections can proceed from the bladder, via the ureters, to the kidneys, where it can cause pyelonephritis, which may lead to irreversible kidney damage, renal failure, and death (Scholes *et al.,* 2005). The presence of bacteriuria among apparently healthy students observed in this study is similar with previous findings in Nigeria and elsewhere; that have demonstrated the presence of bacteria in urine without the occurrence of physical symptoms of UTI (Olusanya *et al.,* 1993; Harrington and Hooton 2000; Ojiegbe and Nworie, 2000; Frank-Peterside and Oguike, 2006; Frank-Peterside and Wokoma, 2009; Farajzadeh *et al.,* 2011; Mwaka *et al.,* 2011; Ngwai *et al*., 2012; Ayoade *et al.* 2013). The prevalence of significant bacteriuria in this study of 13.8% is of high concern especially since the students included in this study did not have complaints suggestive of UTI.

Considering the bacterial organisms isolated from this study, *Escherichia coli* was the most frequent isolate accounting for 37.9%. This is comparable with other studies where *E. Coli* was isolated in 40 – 46% (Moges *et al.,* 2002; Kothari and Sagar, 2008; Mwaka *et al*., 2011). The second most frequent bacteria were *Staphylococcus aureus* with 20.7 % isolation rate. The isolation of *S.aureus* as an uropathogen is not unique to our study. Earlier studies in Nigeria isolated similar rates of *S.aureus* among apparently healthy population (Ojiegbe and Nworie, 2000;Frank-Peterside and Wokoma, 2009; Oluwasegun and Akintunde, 2012; Ayoade *et al.* 2013). However, this finding is in variant with another study with similar population of study where *S.aureus* were not isolated from 531 urine samples analyzed (Kothari and Sagar, 2008). The other isolates in this study included *Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae*. This is in agreement with previous studies where *Klebsiella, Proteus*, and *Enterobacter* were isolated (Kothari and Sagar, 2008; Mwaka *et al*., 2011). Amongst the bacterial agent, *Escherichia coli* is the most frequently incriminated as the causative agents of asymptomatic bacteriuria. Some observational studies suggest that persons with untreated asymptomatic bacteriuria are at increased risk of developing symptomatic urinary tract infections and other complications. Evidence is not conclusive, however, that these clinical outcomes are caused by bacteriuria (especially in the absence of a structural abnormality), or that early treatment

Results in important clinical benefits. This study suggests that periodical health survey is essential for good health of the general population.

The generally similar antibiotic susceptibility pattern of the isolates from the students volunteers indicates that antimicrobial treatment can be achieved in the region with similar drugs. The higher resistance of the isolates to the common and cheap orally administered antibiotics such as amoxicillin, cotrimaoxazole, chloramphenicol, and tetracycline is not surprising because these drugs are more commonly misused, thereby leading to the development of resistance, as previously reported (Ehinmidu, 2005). The higher susceptibility of isolates to other antibiotics such as gentamicin, ofloxacin, and ciprofloxacin was not abnormal, as this has been reported previously (Mbata, 2007). Gentamicin is administered parenterally and, therefore, due to the discomfort of injection, it is less likely to be misused than oral drugs. In contrast, ofloxacin and ciprofloxacin are relatively costly in Nigeria, and this limits their misuse.

Table No.1: The Distribution of Bacteriuria according the Age Group of the Study Population

S.No	Age groups (years)	Number of students sampled	Number of students positive
1	17-19	21	6 (2.4%)
2	20-22	135	21 (8.3%)
3	23-25	76	5 (1.9%)
4	≥ 26	21	3 (1.2%)
5	TOTAL	253	35 (13.8%)

Table No.2: The Frequency and Distribution of Bacteria Isolated from the Students Residing in Different Hostels

Bacteria Isolates	Various Different Student Hostels					
	VH	SH	SH	MH	UH	TOTAL
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
E. coli	3 (10.3)	2 (6.9)	2 (6.9)	1 (3.4)	3 (10.3)	11 (37.9)
K. pneumoniae	2 (6.9)	0 (0.0)	1 (3.4)	1 (3.4)	1 (4.2)	5 (17.2)
P. aeruginosa	1 (3.4)	0 (0.0)	1 (3.4)	0 (0.0)	2 (6.9)	4 (13.8)
S. aureus	1 (3.4)	1 (3.4)	1 (3.4)	2 (6.9)	1 (4.2)	6 (20.7)
P. mirabilis	0 (0.0)	1 (3.4)	1 (3.4)	0 (0.0)	1 (4.2)	3 (10.3)
TOTAL	7 (24.1)	4 (13.8)	6 (20.7)	4(13.8)	8 (27.6)	24 (100.0)

KEY: n = number of isolates, *E. coli = Escherichia coli*, *K. pneumoniae = Klebsiella pneumonia*, *P. mirabilis =Proteus mirabilis*, S. *aureus = Staphylococcus aureus. P. aeruginosa= Pseudomonas aeruginosa*, VH = Victory Hostel, SH = Steve-Jane Hostel, MH = Mediatrix Hostel, UH= Umuguma Hostel

Table No.3: The Antibiotic Susceptibility Pattern of Bacteria Isolated From Female Students of Imo State University Owerri

		Antibiotics									
Bacteria isolated	**Number of Isolates**	**AMX**	**TET**	**COT**	**NAL**	**CIP**	**GEN**	**CRX**	**CHL**	OFX	ERY
E.coli	11	R 100	R 75	R 100	R 40	S 100	S 100	R 30	R 55	S 100	R 57
K. pneumonia	5	R 100	R 80	R 100	R 35	S 100	S 100	R 20	R 35	S 100	R 25
S. aureus	6	R 100	R 100	R 100	R 85	S 100	S 100	R 40	R 60	S 100	R 45
P. aeruginosa	4	R 100	R 50	R 100	R 20	S 100	S 100	R30	R 40	S 100	R 60
P. mirabilis	3	R 100	S 100	R 100	S 100	S 100	S 100	S 100	S100	S 100	S100

KEY: AMX = Amoxycillin, ERY = Erythromycin, TET = Tetracycline, GEN = Gentamicin, COT = Cotrimaoxazole, CHL = Chloramphenicol, OFX = Ofloxacin, NAL = Nalidixic acid, CIP= Ciprofloxacin, CRX = Cefuroxime, R = Resistance, and S = Sensitive.

CONCLUSION

In conclusion, the findings of this study have highlighted the need to include assessment of asymptomatic bacteriuria as part of the medical examination for students, especially new entrants, in tertiary institutions. Ordinarily such students would

not attend a clinic, although as we have observed, they could be carrying an asymptomatic infection. Finally, our study also provides important information on the best choice of antibiotics to treat infections that might arise from these organisms.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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