PREVALENCE OF ASYMPTOMATIC BACTERIURIA IN HEALTHY TERTIARY INSTITUTION STUDENTS IN IJEBU-NORTH OF OGUN STATE, NIGERIA

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Abstract: Asymptomatic bacteriuria, known to be associated with an increased risk of symptomatic urinary tract infection is on the increase. The prevalence differs with respect to age, gender, environment and the presence/absence of genitourinary disorder. This work investigated the burden of asymptomatic bacteriuria and antibiotics sensitivity profile of isolates from University students in Ogun State. Urine samples were collected from asymptomatic students aged 16 to 30 years, culture and antibiotic susceptibility test was done using standard procedure. Pre-tested questionnaire was administered to get vital information about the subjects. All the samples were cultured in different selective media and the plates were incubated accordingly. The isolates were characterized and identified based on the conventional microbiological techniques. Of the 122 students, seventy-two (59.01%) were females and fifty (40. 98%) were males. 54.9% of the subjects had asymptomatic bacteriuria (62.5 and 44% for females and males respectively) while 45.1% of the samples had no sign of growth. The prevalence of asymptomatic bacteriuria was higher (48.9 %) in the age group 20-23 years followed by 37.2% in 16-19 years old. *Escherichia coli* thirty-two (33%) and *Pseudomonas aeruginosa* thirty (31.9%) were the most common bacteria isolated. 52% of the isolates were sensitive to ciprofloxacin. The prevalence of asymptomatic bacteriuria is high among the students studied. University authorities need to include the assessments of bacteria in urine as part of their medical examination for students, and routine evaluation of adolescents for bacteriuria is thereby recommended.

Keywords: Asymptomatic bacteriuria, prevalence, sensitivity testing, students, tertiary institutions

Introduction

Urinary tract infection is one of the most prevalent bacterial infections in humans, and have been reported in all age groups and sex, both at the hospital and community settings (Orrett and Davis, 2003; Chukwu e*t al.*, 2011). UTI has been described as one of the most predominant extra- intestinal bacterial infections that is commonly encountered in medical practice (Ogbukagu *et al.*, 2016). Talha (2018), described UTI as an infection in any part of the urinary system: kidneys, ureters, bladder and urethra but that most infections involve the lower urinary tract – the bladder and the urethra. UTI is also grouped based on the extent of symptoms exhibited by the patients which classified UTIs into symptomatic and asymptomatic (http://www.nhs.uk/conditions/urinary). UTI is caused by both Gram negative and Gram positive bacteria (Behzadi *et al*., 2010; Chukwu *et al*., 2010). The international studies have shown that UTI are very common, therefore one in five adult women experience UTI in her life and is extremely common, clinically apparent, worldwide patient problem (Behzadi *et al.*, 2010; Howes, 2010). It has been observed that annually UTIs account for 8 million visits to doctors' office, 1.5 million emergency room visits and 300,000 hospital admissions in the United States of America (Rahul *et al*., 2009)

Bacteriuria is the presence of bacteria in urine with no evidence of actual tissue invasion (Scottish Intercollegiate Guidelines Network, 2006). Cortes-Penfield *et al.* (2017) described symptomatic bacteriuria as one that will present such symptoms as urgency, dysuria, and frequency with or without lower abdominal pain, fever, and malaise whereas asymptomatic bacteriuria is bacteria in the urine of a patient without any obvious symptoms. Asymptomatic bacteriuria is also described as significant bacterial count in urine usually 10^5or more colony forming units (CFU) per milliliter in a child without symptoms associated with urinary tracts (Chime *et al*., 2018). According to Nicolle *et al*. (2005), absence of UTI symptoms and obstruction is referred to as asymptomatic bacteriuria. Wein *et al.* (2012) reported that bacteriuria may be indication of infection or colonization of the urinary tractor may be due tobacterial contamination occurring during collection of a specimen.

High prevalence of asymptomatic bacteriuria has been attributed to a lot of factors such as sexual intercourse, poor hygienic practices, sharing of towel and clothing, toilet sanitation and maintenance coupled with non-availability of water to clean up the toilets in public places which is common in developing countries (Onifade *et al.*, 2011; Aremu, 2012; Chime *et al.*, 2018). Asymptomatic bacteriuria may lead to symptomatic UTI especially in the presence of factors like urethral reflux disease which may cause pyelonephritis (Lane and Takhar, 2012). The adolescents of marriageable age group is a unique set of individuals who due to their sexually active stage indulge in some risk behavior such as illegitimate sexual act that might predispose them to bacteriuria with its resultant urinary tract infections. They also comprise the reproductive/child bearing age group especially the females who deserves that asymptomatic bacteriuria should be treated in pregnancy in order to avoid the attendant risks to the fetus.

It has been reported that asymptomatic bacteriuria is common in pregnancy thereby subjecting women to increased risk of UTIs (Obiogbolu *et al*., 2009). The anatomical closeness of the female urethra to the vagina makes it liable to trauma during sexual intercourse. Also, the moist environment of the female perineum favours bacterial growth thereby predisposing females to bladder contamination (Ogbukagu *et al.*, 2016). Asymptomatic bacteriuria in adolescents may also signify an underlying structural or functional urinary abnormality which may require specific therapy in order to prevent reoccurrence of infection and subsequent renal damage (Nicolle *et al*., 2005). Since asymptomatic bacteriuria can lead to symptomatic UTI and can also precede asymptomatic UTI, there is need to know the burden of asymptomatic bacteriuria among this population for prompt referral and intervention in order to avoid its associated morbidity and mortality. The aim of this study was to determine the burden of bacteriuria and prevalent etiological agents in this high risk group using students of a tertiary institution in Ogun State, Nigeria.

Materials and Methods

Study site and protocols

This study was conducted at Olabisi Onabanjo University Ago-Iwoye among students from different Faculties. Urine samples were collected from asymptomatic students with age range from 16 – 30 years. The samples were collected within the hours of 8 am – 10 am and the age, gender, Faculty and Department of participants were taken note of. The students were properly guided on how best to collect the urine straight into the bottle without contamination. Those that were not on antibiotics two weeks to the study and that gave their verbal consent were included in the study.

Collection of specimens

Clean catch mid-stream urine samples were collected from consented students onto borate sterile wide mouth 25 ml capacity capped universal containers. This was done by not allowing the bottles to come in contact with external genitalia or adjacent skin. A total of 122 specimens were obtained from students from different departments and faculties. The urine bottles packed in a cooler that contained iced blocks were transported to the microbiology laboratory for microbiological analysis

Culturing and sub-culturing of the urine samples

A loopful of a well- mixed urine samples was streaked onto the surface of already prepared oven-dried media, (MacConkey agar, Centrimide agar, Salmonella-Shigella agar, (Biomark, India) contained in 20 by 20 cm diameter Petric dishes. Incubation was done at 37^{o}C aerobically for 18 – 24 h, after which the colonies were observed and sub-cultured in order to obtain pure cultures. Isolates were Gram- stained in order to determine their characteristics and were identified using classical biochemical reactions profile (catalase, coagulase, oxidase, citrate, indole, H_2S, and gas). Growth of $\geq 1\times 10^5$ CFU/ml was taken as significant bacteriuria but for *Staphylococcus saprophyticus* growth of between $> 1\times10^3$ and $< 1\times 10^5$ CFU/ml was regarded as significant (Murray *et al*., 2003).

Antibiotic susceptibility test

Antibiotic sensitivity pattern of the significant isolates were done using the disc diffusion method as described by the Clinical Laboratory Standard Institute (2012) using diagnostic sensitivity test agar (Biomark, India) and single discs antibiotics. A colony of pure isolates was streaked on sterile Mueller Hinton agar plates (Biomark, India) aseptically using sterile inoculating wire loop. A sterile forcep was used to place the antibiotic sensitivity disc on the surface of the culture plates. The plates were incubated aerobically at 37^{0}C for 24 hrs. The zone of inhibition was measured to the nearest millimeter using a slide gauge. The zone of inhibition in millimeter of each of the antibiotics to the isolate was then compared with the standard zone diameter in interpretative chart (CLSI, 2012). This was used to report whether the isolate is sensitive, intermediate or resistant to the antibiotics. The following antibacterial agents: ciprofloxacin 5 µg, novabacin 10 µg, ampicillin 10 µg, trimethoprin-sulphamethazole 10 µg were tested.

Statistical analysis

The data obtained were analyzed using the software package for Social Science (SPSS) version 20 for windows. Data were summarized using frequency, percentage, mean and standard deviations.

Results and Discussion

Out of 122 samples collected (72 females and 50 males) from adolescents/adults aged 16-30 years, significant bacteria growth was observed in 67 samples (45 females and 22 males) with a prevalence rate of 54.9 % (67/122) whereas 55 samples 45.1% (55/122) showed no signs of growth (Tables 1 and 2). However, 94 bacterial isolates were identified, with Gram negative bacteria 88.3% (83/94) being the highest in occurrence as against Gram positive ones 11.7% (11/94). *Escherichia coli* 31(33%) was the most predominant Gram negative organism isolated while *Staphylococcus saprophyticus* was the most Gram positive organism isolated comprising 90.9% (10/11). Other Gram negative bacteria isolated were *Pseudomonas aeruginosa* 30(31.9%), *Klebsiella pneumonia* 13(13.8%), *Proteus mirabilis* 5(5.32%) and *Enterococcus faecalis* 4 (4.3%) (Table 3).

Table 1: Number of urine samples collected according to age and sex

Age group	Males	Females	Total
16-19	12	33	45
20-23	26	30	56
24-27	11	08	19
28-30	01	01	02
Total	50	72	122

Table 2: Distribution of Samples with Growth and without growth according to gender

Sex	No. Growth	Growth	Percentage Growth (%)
Male	28	22	32.8
Female	27	45	67.2
Total	55	67	100

Table 3: Frequency and percentage of occurrence of isolated bacteria

Organisms	Frequency	Percentage
E. coli	31	33
K .pneumoniae	13	13.8
S. saprophyticus	10	9.6
S. epidermidis	01	1.1
P. aeroginosa	30	31.9
P. mirabilis	05	5.3
E. faecalis	04	4.3
Total	**94**	**100**

All isolated bacteria were more common in females 67/94 (71.3 %) than in males 27/94 (28.7%), and the age group 20-23 had the highest occurrence (48.9%) of the isolated bacteria especially *E. coli* and *P. aeruginosa* in which 15 (32.6 %) and 13 (28.3 %) respectively were observed. The 16-19 age range was the second in terms of number of bacterial organisms isolated 35 (37.2 %) with *P. aeruginosa* predominating 16 (45.7 %) (Table 4). Age range of 28-30 and 24- 27 had the least number of isolated bacterial as it was only a total of 2 (2.1 %) and 11(11.7%) isolates, respectively were recovered. Of the 84 isolates subjected to sensitivity testing: 24, *E. coli*, 28, *P. aeruginosa*, 13, *K. pneumonia*, 10, *S. saprophyticus*, 01, *S. epidermidis*, 04, *E. faecalis* and 04, *P. mirabilis*, 76 (90%) were sensitivity to the 4 antibiotics tested. Out of the 24 isolates of *E. coli*, 23 (27%) were sensitive to all the tested antibiotics with 10 (45%) being sensitive to ciprofloxacin and 6 (25%) to trimethoprin- sulphamethazole. Of the 28 isolates of *P. aeruginosa*, 13 (15% were sensitive to the tested antibiotics with 11 (25%) being sensitive to ciprofloxacin. However, 44 (52%) and 20 (24%) of all the tested isolates were sensitive to ciprofloxacin and trimethoprin-sulphamethazole, respectively (Table 5).

Table 4: Distribution of the isolated bacteria according to age and gender

Age group	*E. coli*		*P. aeruginosa*		*K. pneumonia*		*P. mirabilis*		*Non-coagulase S. spp.*		*E. feacalis*		Total
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
16-19	0	7	5	11	1	3	1	3	2	1	0	1	35
20-23	3	12	5	8	2	5	0	1	2	5	1	2	46
24-27	3	4	0	1	1	1	0	0	0	1	0	0	11
28-30	1	1	0	0	0	0	0	0	0	0	0	0	2
Total	7	24	10	20	4	9	1	4	4	7	1	3	94

Table 5: Mean antibiotic Susceptibility patterns and Zones of inhibition of tested strains of bacterial organisms

Antimicrobials	Disk Potency	Sensitivity of standard strains (mm)	Zones of inhibition (mm)						
			E.C	P.A	KP	SS	S.E	E.F	P.M
CPX	5 µg	≥21	24.95	13.50	25.53	24.50	31.00	28.50	17.25
NV	30 µg	≥17	3.12	1.57	8.69	5.90	18.00	10.75	–
AM	10 µg	≥17	4.87	0.89	5.07	4.40	10.00	3.00	–
SXT	10 µg	≥16	7.25	0.89	10.69	8.70	23.00	16.25	–

CPX – Ciprofloxacin, NV:- Nobacin, AM:- Ampicillin, SXT:- Timethoprin-sulphurmethazole, E.C:- *Escheriscia coli*, P.A:- *Pseudomonas aeruginosa* , KP:- *Klebsclia pneumonia*, SS:- *Staphilococcus saprophyticus*, SE:- *Staphilococcus epidermis*, EF: *Enterobacter feacalis*, PM:- *Proteus mirabilis* Standard Strains- acceptable inhibitory zones for each antibiotics according to Clinical and Laboratory Standard Institute (2012)

Table 6a: Summary and interpretation of the antibiotic sensitivity test

Bacteria Isolates	Ciprofloxacin (5 ug)			Novabacin (30 ug)			Ampicillin (10 ug)			Timethoprin-Sulphurmethazol (10 ug)		
	S	I	R	S	I	R	S	I	R	S	I	R
E. coli	10	8	6	3	-	21	4	0	20	6	0	18
P. aeruginosa	11	0	17	1	-	27	0	0	28	1	0	27
K. Pneumonia	7	2	4	1	-	13	1	1	11	6	0	7
Non-coagulase *Staphylococcus*	10	0	1	1	-	11	0	0	11	5	0	6
E. faecalis	4	0	0	1	-	3	0	0	4	2	1	1
P. mirabilis	2	0	2	0	-	4	0	0	4	0	0	4
Total	44	10	30	7	-	79	5	1	78	20	1	63

R = Resistance, I = Intermediate, S = Sensitive

Table 6b: Percentage susceptibility of the antibiotics across the isolates

Antibiotic disc	Sensitive	Intermediate	Resistance
Ciprofloxacin (5 ug)	53.3	11.6	36
Novabacin (30 ug)	8.1	-	91.9
Ampicillin (10 ug)	5.8	1.2	93
Timethoprin-Sulphurmethazole (10 ug)	23.3	1.2	75.5

UTI is the major cause of morbidity both in hospital and community settings and affects both out and in Patients. It is one of the infections that could lead to chronic kidney disease and also the most common health care associated group of bacterial infections affecting humans in Africa (Omigie *et al.*, 2009).

The prevalence rate of 54.7 % for asymptomatic bacteriuria observed in this study is however similar to what was reported in other studies in Nigeria although among primary and secondary school children. Azubuike *et al*. (1994) and Alo e*t al*. (2012) obtained 30 and 48% prevalence among primary school children in Awka and rural primary school children in Ebonyi State respectively. However, a prevalence of 14.25, 14 and 14.5% were reported by Chime *et al*. (2018), Akor *et al*. (2009) and Mbakwem-Aniebo *et al*. (2006) in their studies among primary school children in Enugu, Jos and Port Harcourt respectively. A much lower prevalence had been reported both within and outside Nigeria (Nwokocha *et al*., 2014), in Enugu reported 9.7% prevalence while Yayli *et al*.(2003) in Turkey reported prevalence rates of 0.12% out of 10, 289 school children they screened compared to 122 students in the present study.

The high prevalence of ASB in this study could be attributed to low sample size of 122, and only one university was used in the study which could bring about poor distribution of subjects. This is contrary to that of Chime *et al*. (2018) and Nwokocha *et al*. (2014) who enrolled 400 and 628 samples, respectively. The high ASB rate (48%) observed in Akor *et al.* (2009) was attributed to the fact that a diagnostic cut-off of $>10^4$ CFU/ml was used as their definition of significant bacteriuria instead of $> 10^5$ CFU/ml and also, the study was carried out among children in rural areas who are known to have low level of hygiene and poor health consciousness. Moreover, the 30% ASB rate documented in Azbuike *et al*. (1994) was also due to the low sample size of 200 which did not represent the appropriate percentage of the total population (33,125 pupils) of primary school children in Awka and one primary school was employed (Ujunwa *et al.*, 2013). The reason for the 0.12% prevalence reported by Yayli *et al.* (2000) could be due to improved level of hygiene in developed countries such as Turkey unlike what is obtainable in developing countries like Nigeria.

It would have been expected that low prevalence rate of ASB should be observed among students of tertiary institutions than the secondary school counterpart as it had been reported that as age increases, there tend to be decline in the prevalence of ASB among the late adolescents due to improved level of hygiene consciousness Nwokocha *et al*. (2014) but the reverse

is the case. A prevalence of 71.3% which was observed among the females was in agreement with the reports of so many authors (Onifade *et al.,* 2011; Nwokocha *et al*., 2014; Ogbukagu *et al.,* 2016). However, this finding contradicts the reports of Ngwai and Bakare, (2012). The higher incidence in female than male could be due to the shortness and closer of the female urethra to the anus (Nwankwo *et al.,* 2017), and /or lack of the bacteriostatic properties of prostatic secretions (Onifade *et al.,* 2011). It could be that the low level of hygiene and habit of long withholding of micturition among female leads to easy colonization of the tract. This however, stressed the need for serious health education and improved personal hygiene among the students especially in the schools where there are poor toilet facilities and non-availability of constant water supply. Another factor could be the unhygienic practices of cleaning the anus and vagina from the back to the front after defecation and urination which leads to autoinfection. The low prevalence in male (28.7%) may be due to the antibacterial property of prostatic fluid and high rate of circumcision in males of southwestern Nigeria as it had been reported that non circumcision predisposes males to ASB (Ogbukagu *et al*., 2016). This finding is in disagreement with the report of Wennerstrom *et al.* (2000) who observed higher ASB rate in males than in females which they attributed to high incidence of un-circumcision among the male subjects in their study.

The age group 20-23 years (49%) followed by 37.2% in 16-19 years in this study had the highest rate of ASB is in line with the reports of several authors (Oluremi *et al.,* 2011; Oladeinde *et al.,* 2011; Nwokocha *et al*., 2014; Ogbukagu *et al*., 2016). Infection is during youth, adulthood and teenager due to their vulnerability to increased sexual activities which predisposes them to infection (Kirby *et al.,* 2007).

The most common pathogen observed in this study was *Escherichia coli*, followed by *Pseudomonas aeruginosa*, coagulase negative *Staphylococci*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Enterococcus faecalis*. This observation was in line with the reports of Oluyemi *et al.* (2012) and Mbakwem-Aniebo and Ene, (2006) whose findings were that *E. coli*was the most common isolates among school children. In same vein, studies done by Musa-Aissien *et al*. (2003), Asinobi *et al*. (2003) and Chime *et al*. (2018) revealed that *E. coli* was the commonest bacterial isolate in urine. Some studies conducted outside Nigeria also reported *E. coli* as the most common isolate (Saleh *et al.,* 2003; Sawalha and Taha, 2009). *Escherichia coli* being the most prevalent bacteria isolated could reflect its origin which is usually from the microflora of the intestine and perineum (Elder, 2004). According to Oelschlaeger *et al.* (2002), *E. coli* virulence factors such as toxin and fimbriae contain adhesion at the tip which aids in attachment to the uroepithelial cells. The adhesion factor prevents the organism from being washed off with voiding, and enables it to persist and cause infection (Chime *et al*. 2018).

However, this finding does not agree with most of the recent works of several authors who documented different bacteria as the main causative agents (Anochie and Eke, 2003; Muoneke *et al*., 2012 Alabi *et al.,* 2014); all reported different Klebsiella species as the commonest bacteria implicated in asymptomatic bacteriuria and urinary tract infections. In contrast too, Alo*et al.* (2012) and Otajevo and Eriagbo (2014) reported S*taphylococcus aureus* as the most common occurring bacteria in school children. Staphylococcus which was the predominant bacteria in their studies could reflect the unhygienic nature of the environment where the subjects reside as it had been reported that human beings harbor Staphylococcus on their skin, and that the organism is a good indicator of the standard of hygiene and widely distributed in the environment among the unhygienic ones (Aiyegoro *et al*., 2007). The reason why staphylococcus was the most common in their studies could be due to the fact that their studies were done in a rural community where it is likely that the subjects might live in an unhygienic environment unlike this present study that was carried out in university environment.

In this study, it was observed that the antibiotics sensitive profile of the isolates revealed high sensitivity to ciprofloxacin. This is consistent with the work of Musa-Aissien *et al.* (2003) in Benin, Geofrey *et al.* (2013), Kline *et al*. (2010) and Cai *et al.* (2011) who separately documented high sensitivity of urinary isolates to ciprofloxacin. Similarly, Aiyegoro *et al.* (2007), Kolawole *et al.* (2009) both in IIe-Ife, Brown *et al.* (2004) in Ibadan, Akinbami *et al.* (2013) and chime *et al.* (2018) in Enugu; all reported that the quinolones are the most potent of all the antibiotics in their study. The high sensitivity of this quinolone class may be due to the fact that they are relatively new drugs which could not have been extensively used, thereby discouraging the development of resistance by the pathogens. Arora and Arora, (2008), attributed the high sensitivity of ciprofloxacin to the fact that it has broad spectrum of activity.

All the tested isolates in this study displayed 93, 92 and 76% resistance to ampicillin, novabacin and trimethoprin-sulphurmethazole, respectively. Rabasa and Shattima, (2002), Brown *et al.* (2004) and Chime *et al.* (2018) reported the resistance of nitrofurantoin, cotrimoxazole and amoxicillin by the urinary pathogens. The habit of self-medication, use of inferior and adulterated drugs including under and over use may probably be responsible for the resistance. Boekitwetan *et al.* (2012) reported that resistance exhibited by the isolates against the other antibiotics might be genetic or acquired features which allow the organism to resist the action of antibiotics.

Conclusion

Bacteriuria remains an important problem that cuts across all age groups and both genders. The prevalence of ASB in the studied area was 54.7 % and is commoner in adolescent aged 20-23 years. The most predominant organism was *Escherichia coli.* Up to 50 % of the isolates were sensitive to ciprofloxacin but there was in-vitro resistance to novabacin, ampicillin and trimethoprin-sulphurmethazole. It was therefore concluded that there is need for steady, periodic monitoring of the pattern of organisms responsible for asymptomatic bacteriuria and their antibiotic sensitivity profile in various states in order to know the changing trends. Ciprofloxacin could be considered for empiric treatment of urinary tract infection among the population in the studied area. However, their use should be guided as much as possible by antibiotic sensitivity studies.

Conflict of Interest

No conflict of interest was declared by the authors.

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