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Full Length Research Paper

Prevalence of Asymptomatic Bacteriuria amongst Residential Female Students of a Tertiary Institution in Benin City Nigeria.

Idemudia, Nosakhare Lawrence[1*]; Okoro, Azuka Vivian[2] and Ohiengbomwan, Tola Onaiwu[3]

[1]-Department of Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.
[2]-Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria.
[3]-Department of Health Services, Redeemer's University, Ede, Osun State, Nigeria.

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Corresponding Author:
Idemudia, N. Lawrence
Department of Medical Laboratory Services, University of Benin Teaching Hospital, Nigeria.

Abstract
Asymptomatic bacteriuria is believed to be more common amongst young females even though much is not known about its risk factiors and pathogenesis, its ability to result in symptomatic urinary tract infection is a huge possibility thereby causing grave public health challenges. This study was carried out to determine the prevalence of asymptomatic bacteriuria among the female students of the University of Benin, Ugbowo, Benin City, residing in the Halls of Residence within the University. A total of Two Hundred (200) female students who had neither abnormalities of the urinary tract nor shown any sign of urinary tract infections were recruited for the study and their urine samples were obtained and analysed microbiologically. Bacteria count was done on plate count agar (PCA) for all urine samples collected which were also innoculated on blood agar and cysteine lactose electrolyte deficient (CLED) agar based on standard methods. The antibiotic susceptibility pattern for the isolated bacteria was determined. Bacteria growth was observed in 54% of the samples, while there was no growth in 46% of the samples. Of the 108 samples with bacteria growth, only 46 significant bacteriuria (>10^5CFU/mL) was recorded. The bacterial organisms isolated were Escherichiacoli, Proteus mirabilis, Providencia species and Staphylococcus aureus. All the bacteria isolates were resistant to Ceftazidine and Cefuroxime while Ofloxacin was the most sensitive (89.1%) followed by Ciprofloxacin (78.3%), Nitrofurantoin (69.6%), Gentamycin (60.9%), Augmentin (43.5%) and Cefixime (32%). This study revealed that some bacterial isolates could cause urinary tract infections (UTI) without any notable clinical symptoms hence the need for health education especially on the need for improved personal hygiene among the female students and inclusion of urine culture in medical checks upon admission.

Keywords: *Asymptomatic, bacteriuria, antibiotic susceptibility*

Introduction

Asymptomatic bacteriuria (ASB) is a variety of urinary tract infection (UTI) characterized by the presence of significant amount (up to 10^5 cfu/ml) of bacteria in the urine. Though more common in females and the elderly, ASB does not have age specificity as it can occur in both infants and adult and even in up to 6% of healthy individuals (Ghandhi, 2006). It is usually associated with increased risk of retarding intra-uterine growth and low birth weight in infants (Hazhir *et al.*, 1976; Schultz *et al*., 1991) and development of cystitis or pyelonephritis especially in pregnant women where it can also cause preeclampsia (Kass, 1970).

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is perhaps the single most common bacterial infection of mankind (Johnson and Stamm, 1989). The normal genitourinary tract is sterile, apart from the distal urethra (Ebie *et al.*, 2001). Though the urinary tract is normally sterile, bacteria that generally ascend from the peri-anal area reservoir may cause UTIs, this is more common in females (Gupta *et al.*, 2001; Colgan and Williams, 2011). Bacteria in the urinary tract may remain asymptomatic or cause irritative symptoms such as frequency and urgency of urination. If untreated, the infection may ascend to the upper urinary tract and produce fever, chills and flank pain. Bacterial entry into the blood stream (bacteremia) is associated with severe morbidity, including sepsis and death (Mignini *et al.*, 2009). Stamm and Hooton, (1993) referred to UTI as a clinical (symptomatic) or subclinical (asymptomatic) diseases that may involve just the lower tract or both the lower and upper tract. Infection may involve single site such as urethra (urethritis), prostrate (prostitis), bladder (cystitis) and kidney (pyelonephritis). UTI is one of the most common diseases occurring from neonates up to geriatric age groups with 40-50% of adult women having a history of at least one UTI (Johnson and Stamm, 1989). The presence of bacteria in urine without symptoms is termed asymptomatic bacteriuria (Ojiegbe and Nworie, 2000) this occurs following ascension of bacteria up the urethra into the bladder, sometimes with subsequent ascension to the kidneys. Bacteria

isolated from the urine of patients with asymptomatic bacteriuria usually originate as colonizing flora of the gut, vagina or peri-urethral area (Nicolle, 2003). It is more common in women, in the elderly, in residents of long-term care facilities, and in people with bladder catheters, spinal cord injuries and diabetes. People with a long-term catheter consistently show bacteriuria while chronic asymptomatic bacteriuria occurs in about 50% of the population in long-term care (Timothy *et al*., 2006). Symptomatic bacteriuria is an iceberg of total bacteriuria (Anayet *et al.,* 2007). The microbiological count is usually between 10^2 and 10^4 colony forming unit per milliliter (Franz and Horl, 1999). The risk factors identified with high prevalence of UTIs in young adult females include sexual intercourse, spermicide-based contraception and a history of UTIs (Gupta *et al.,* 2001). Urinary tract infection is characterized by fever, flank pain and tenderness in addition to significant bacteriuria. Other symptoms may include nausea, vomiting, frequent urination, urgency, dysuria, premature and low birth weight (Schultz *et al*., 1991). The most common bacteria implicated in the pathogenesis of urinary tract infection include *Escherichia coli* (Ebie *et al*., 2001), *Staphylococcus aureus* (Ugbogu *et al*., 2010), *Pseudomonas aeruginosa, Klebsiella aerogenes. Enterobacter* species and *Proteus* species (Kolawole *et al.,* 2009).

The aim of this research is to investigate the prevalence of asymptomatic bacteriuria (ASB) amongst residential female students in the University of Benin, Benin-city, to identify the bacterial agents implicated in the infection and their susceptibility to various antibiotics.

Material and methods

Study area and participants

The study was conducted at the University of Benin, Ugbowo Campus, Benin City, on female students residing in the hostel facilities of the university. University of \Benin is one of the foremost federal higher institutions in Nigeria which play host to students drawn from almost all the states in Nigeria. The University has over 10,000 students both at the undergraduate and post graduate levels with most of them residing in the halls of residence.

Sample size was determined using the formula: $N = (z^2pq)/d^2$ (Jaykaran and Tamoghna, 2013).

Where: N = desired sample size when population is greater than 10,000,
Z= standard normal deviation, usually set at 1.96 or simply 2.0 (at 95% confidence interval),
P = proportion of the target population estimated to have a particular characteristics
q=1.0-p
d= degree of accuracy desired, usually set at 0.05.

Assuming ASB prevalence of 15% = 0.15 (Wogu and Ogbebor 2011)

From calculation using $N = (z^2pq)/d^2$
$N= (1.96)^2 \times 0.15 \times (1.0-0.15)/ (0.05)^2$
N = 196.

Therefore, a total of Two Hundred (200) asymptomatic female students (100 each from Queen Idia and Tinubu Halls) were randomly selected. Students who were on antibiotic treatment two weeks prior to the collection of urine, those seeing their menstruation cycle, those who previously had urinary tract infection (UTI) and those who declined informed consent were excluded from this study.

Collections of urine samples

Information on age, sexual activity and use of antibiotics for the past two weeks was obtained with the aid of a well-structured questionnaire detailing the scope and purpose of the study and column for informed consent. The students were then adequately educated on how to take mid-stream sample of their urine into a sterile capped, dry, wide-necked, leak-proof and labeled sample universal bottles which contained boric acid as preservative.

Media used

Plate Count Agar, CysteineLactose Electrolyte Deficient (CLED) agar, blood agar and nutrient agar were used for bacteria count, culturing and antibiotic susceptibility testing accordingly as described by Chessbrough (2006).

Urine culture and bacteria count

Before inoculation, the urine samples were properly shaken to ensure even distribution of microorganisms. The urine samples were then cultured using a sterilized standard loop with internal diameter of 3mm, calibrated to hold 0.01 ml of urine onto Plate Count Agar (PCA), blood agar and Cysteine Lactose Electrolyte Deficient (CLED) agar. Inoculated plates were incubated at 37°C aerobically overnight.

Identification and counting of bacterial isolates

The different bacteria colonies were identified on the basis of their colony morphology (color, growth size and growth pattern). Bacterial counts were determined by dividing the colony count (colony on PCA) by the volume of urine (0.01ml) inoculated. Significant UTI (significant bacteria growth) was determined by a bacterial count greater than 1 $x10^5$cfu/ml, while a bacterial count less than 1 x 10^5cfu/ml was regarded as insignificant UTI (insignificant growth).

Antimicrobial susceptibility test (ast)

The susceptibility of the isolates to selected antimicrobial agents was determined by the Kirby Bauer methodusing antibiotic-impregnated paper discs containing the following antibiotics: gentamycin, ofloxacin, cefixime, augmentin, nitrofurantoin, ciprofloxacin, ceftazidime and cefuroxime. For antibiotic sensitivity test, a standard wire loop (3mm in diameter) was used to collect a portion of the bacteria colony. This was emulsified in peptone water and the turbidity was adjusted to the equivalent of 0.5 MacFarland standards. This was then poured into nutrient agar culture and excess fluids were drained. The sensitivity disk (multi-disk) was placed on the media. The plates were then incubated overnight at 37°.

Results

A total of Two hundred female students of the University of Benin, residing in the halls of residence within the university were recruited into the study and their urine samples were collected analyzed microbiologically. Table 1 shows the bacterial count of urine samples of study participants and it reveals that 46 students out of the 200 participants (23%) had ASB since the bacteria count obtained from their urine where $\geq 10^5$CFU/mL, 37.5% had no bacterial growth while the others had insignificant bacteriuria. Bacterial counts according to the age group of students are shown in table 3 which reveals that 57% of the cases of ASB were amongst students within the age range of 19-22years followed by 23-26years group. The bacterial organisms isolated as shown in table 3 indicates that *Proteus mirabilis* has the highest prevalence as the causative agent of ASB among the female students of the University of Benin with 36.9%, followed by *Staphylococcus aureus* (32.6%), *Escherichia coli* (28.3%) and *Providencia* spp having the lowest prevalence (2.2%). Table 4 shows the bacteria isolated from the urine samples with ASB and their antimicrobial susceptibility pattern which highlight the fact that all the bacteria isolates were resistant to Ceftazidine and Cefuroxime while Ofloxacin was the most sensitive (89.1%) followed by Ciprofloxacin (78.3%), Nitrofurantoin (69.6%), Gentamycin (60.9%), Augmentin (43.5%) and Cefixime (32%).

Table 1: Bacterial count of urine samples

Bacterial count(cfu/ml)	Number (%)
Nil	75(37.5)
$10-10^3$	27(13.5)
10^3-10^4	52(26.0)
$\geq 10^5$	46(23.0)
TOTAL	**200 (100)**

Table 2: Age group of the students and incidence of Asymptomatic Bacteriuria

Age Group (Years)	Number sampled (%)	Number with Asymptomatic Bacteriuria (%)
<15	31 (15.5)	1(2)
15-18	81 (40.5)	6(13)
19-22	63 (31.5)	26(57)
23-26	18 (9.0)	11(24)
>26	7 (3.5)	2(4)
TOTAL	**200 (100)**	**46(100)**

Table 3: Bacterial organisms isolated from urine samples

Organisms	Numbers of Cases in which it was isolated (%)
Escherichia coli	13(28.3)
Proteus mirabilis	17(36.9)
***Providencia* spp**	1(2.2)
Staphylococcus aureus	15(32.6)
Total	**46(100)**

Table 4: Antibiotic Susceptibility Pattern of the Clinical Isolates Obtained from the Urine Samples.

		Antibiotics							
Isolates	**Total no.**	**OFL**	**AUG**	**NIT**	**CPR**	**CAZ**	**CRX**	**GEN**	**CXM**
Escherichia coli	13	13(100%)	10(0%)	7(67%)	13(100%)	0(0%)	0(0%)	8(33%)	**5(33%)**
Proteus mirabilis	17	13(100%)	0(0%)	13(100%)	15(100%)	0(0%)	0(0%)	13(100%)	**11(33%)**
***Providencia*spp.**	1	1(100%)	0(0%)	0(0%)	1(100%)	0(0%)	0(0%)	1(100%)	**0(0%)**
Staphylococcus aureus	15	14(57%)	10(0%)	12(28%)	7(100%)	0(0%)	0(0%)	6(86%)	**0(0%)**
Total (%)	**46**	**41(89.1%)**	**20(43.5%)**	**32(69.6%)**	**36(78.3%)**	**0(0%)**	**0(0%)**	**28(60.9%)**	**16(34.8%)**

KEY:
OFL: Ofloxacin *AUG: Augmentin* *NIT: Nitrofurantoin*
CPR: Ciprofloxacin *CAZ: Ceftazidime* *CRX: Cefuroxime*
GEN: Gentamycin *CXM: Cefixime*

Discussion

This study was carried out to determine the presence of asymptomatic bacteriuria among 200 female students of University of Benin, residing in the halls of residence that neither had abnormalities of the urinary tract nor symptoms of UTI. The prevalence of ASB was observed in 23% of the population. Prevalence of 9.5% of asymptomatic bacteriuria was reported by Obirikorang *et al.* (2012). Varying prevalence rates of ASB in females have been reported by Hazhir (2007) reporting a prevalence of 6.1%, Turpin *et al.* (2007), reported a prevalence of 8.4% while Moghadas and Iraijian (2009) reported a prevalence rate as low as 3.3%. High incidence of ASB has been attributed to low social- economic group (Timothy *et al*., 2006). Ayoade *et al*. (2010), attributed incidence of ASB in females as a result of several clinical factors including hormonal effects, behavioral pattern and anatomical differences. The incidence of ASB in this study was observed to be more within the age bracket of 19-22yrs which comprise of 57% of the ASB cases in this study. This may be attributed to the fact that females within this age group are known to be very active sexually as they comprise young adults who like to try new things especially a new way of life as most of them may be leaving home for the first time.

The present result showed a 23% occurrence of significant ASB among the total of 200 samples examined as illustrated in (Table 1), *Proteus mirabilis* has the highest prevalence (36.9%) while *Providencia* sp has the least prevalence (2.2) among the bacterial species isolated as shown in (Table 3). Most bacteria isolated from the urine samples were *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis*, which is similar to the reports of Ebie *et. al.,* 2001 and Ugbogu *et. al.,* 2010. Bacterial species isolated from the cases of ASB in this study showed varying susceptibility pattern to eight (8) antibiotics in this order: Ofloxacin was the most sensitive (89.1%) followed by Ciprofloxacin (78.3%), Nitrofurantoin (69.6%), Gentamycin (60.9%),Augmentin (43.5%) and Cefixime (32%) while all the isolates were resistant to Ceftazidime and Cefuroxime as shown in (Table 4). The antibiotic sensitivity patterns from this study showed that some of the *Escherichia coli* were sensitive to nitrofurantoin and gentamycin which agrees with the research carried out by Obirikorang *et. al*. (2012), who had a similar report.*Proteus mirabilis* and *Escherichia coli* followed by *Staphylococcus aureus* were sensitive to gentamycin which agrees with the report of Boye *et al*. (2012). *Proteusmirabilis* was sensitive to Ofloxacin, Ciprofloxacin and Gentamycin. *Escherichia coli* was resistant to Augmentin and sensitive to Ciprofloxacin which contradicted the report of Frank-Peterside and Wokoma (2009) who reported that *Escherichia coli* was sensitive to Augmentin but resistant to Ciprofloxacin, and that *Proteus mirabilis* was resistant. *Proteus mirabilis* and *Escherichia coli* were sensitive to ciprofloxacin and nitrofurantoin which agrees with study carried out by Dellit *et. al.* (2007).*Staphylococcus aureus* was sensitive to ciprofloxacin and gentamycin which agrees with the study carried out by Frank-Peterside and Wokoma, (2009). *Proteus mirabilis* and *Staphylococcus aureus* were sensitive to gentamycin which contradicted the study carried out by Boye *et al*. (2012). This may be attributed to differences in host susceptibility to these pathogens as a result of biological and environmental factors that encourage biodiversity in host, pathogens, vector and social factors such as personal effort in controlling diseases (Boye *et. al*., 2012).

Conclusion

Asymptomatic bacteriuria is prevalent (23%) among female students of the University of Benin with *Proteus mirabilis* implicated as the most prevalent causative agent while *Providencia* sp has the least prevalence. Personal hygiene should be encouraged while self-medication which could lead to antibiotic resistance should be discouraged. There is also a great need for health education and creation of awareness on the significance of personal hygiene and cleanliness around the urogenital and anal area as a way of preventing faecal contamination of the urinary tract in females. However, if asymptomatic bacteriuria is suspected at any time, it should be investigated and treated as proper treatment could avert more serious problems of the urinary tract.

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