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ASYMPTOMATIC BACTERIURIA AMONG STUDENTS IN MICHAEL OKPARA UNIVERSITY OF AGRICULTURE, UMUDIKE.UMUAHIA.ABIA-STATE.NIGERIA

[1]Mbajiuka Chinedu S and [2]*Enya Emmanuel

[1]Department of Microbiology; Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

[2]Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

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***Correspondence for Author**
Enya Emmanuel
Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

ABSTRACT

Asymptomatic bacteriuria is said to be a situation when bacteria are present in urine in the absence of clinical signs and symptoms of urinary infection in the hosts. The microbiological definition is having greater than or equal to 10^5cfu/ml of same organism(s) in a urine specimen. This study investigated the prevalence of asymptomatic bacteriuria in female students and the antibiotic sensitivity pattern of bacterial isolates was determined. 80 urine samples were studied. Mid-stream urines were collected and examined microscopically and cultured. Samples were cultured on nutrient agar, MacConkey and Blood Agar and incubated at 37^OC for 24hrs. Isolates were tested against antibiotics which included Ampicillin, Cotrimoxazole, Ofloxacin, Augmentin, Ciprofloxacin, Gentamicin, Tetracycline and Chloramphenicol by disc diffusion method. The total occurrence of asymptomatic bacteriuria was 48.7% while the isolates were *E. coli* representing 22.5% of the isolates. Others were *S. aureus* 12.5%, *Klebsiella spp* (5.00%); *Proteus spp* (5.00%) and *Streptococcus spp* (3.75%). A negative test for the nitrite and leucocyte esterase pad on the dipstick test was not used to rule out an infection as bacterial growth on culture media was also demonstrated for such samples. Positive tests for the nitrite and leucocyte esterase pad from the urine dipstick analysis however required further confirmation through urine culture. Ofloxacin was the most active antibiotic as it achieved a susceptibility rate of 88.9% against *E. coli* while Tetracycline and Ampicillin recorded the least. The incidence rate of 48.7% reported in this study should be of great concern because asymptomatic bacteriuria predisposes patients to UTI.

KEYWORDS: Bacteriuria, Pyuria, Genitourinary, Asymptomatic, Pathogens.

INTRODUCTION

The term bacteriuria means the presence of bacteria in urine and it is taken to be significant when urine contains a bacterial count of 10^5cfu/ml in voided mid stream urine, aseptically collected from an individual without apparent symptom of urinary tract infection (Forbes *et al*, 2002; Ophori *et al*; 2010). Asymptomatic bacteriuria is bacteriuria without the classical symptoms of urinary tract infection (fever, frequent urination; painful urination) the importance of asymptomatic bacteriuria is that it is a major risk factor for the development of UTI (Patterson and Andriole, 1997).

Bacterial infection of the urinary tract in human are the most frequent bacterial disease, affecting out patients, hospitalized patients and apparently healthy populations (Piatti *et al*; 2008). The frequency and natural history of asymptomatic bacteriuria vary for different populations and is more common in females than in males by virtue of the shortened urethra.
Asymptomatic bacteriuria can occur in both infants and adults but is seen more frequently in females than in males and is a major concern in UTI (Nurullaev, 2004). This is because under favorable conditions, asymptomatic bacteriuria progresses to symptomatic (clinical) UTI (Harrington and Hooton, 2000). Factors such as shortness of urethra, estrogen deficiency, use of contraceptives, diabetes, sexual activity, easy contamination of urinary tract with fecal Flora (Gupta *et al*; 2001), obstructing lesions, and genetic factors such as blood group secretor status, increase the likelihood of women contracting a UTI.

The clinical significance of asymptomatic bacteriuria has been however controversial because the widespread use of the quantitative urine culture provided a reliable means for identification of the causative bacteria. This has led to question whether bacteriuria, in the absence of symptoms, leads to complications of urinary infection. Adverse outcome, of concern have included the short-term complications of symptomatic lower tract infection and longer-term complications, such as renal failure. Alternatively, asymptomatic bacteriuria may be beneficial. Colonization of the genitourinary tract by a virulent organism could prevent infection with more virulent organisms, through competition for nutrients, or receptor sites, by eliciting a cross host immune or inflammatory response or by other mechanisms.
Bacteria that colonize the urinary tract (which include organs that collect and store urine and release it from the body: kidneys, ureters, bladder and urethra) may ascend towards the bladder to cause cystitis, which is usually associated with the classic symptoms of UTI. UTI

can proceed from the bladder, through the ureters, to the kidneys where it can cause pylonephritis, which may lead to irreversible kidney damage and renal failure.

The most bacteria etiologic agents in asymptomatic bacteriuria are mostly members of the Enterobacteriaceae *Klebsiella pneumonia, Escherichia. Enterococcus faecalis, Streptococcus agalactiae; Staphylococcus and Streptococcus pyogenes* (Ophori *et al*; 2010) are also implicated with *E. coli* being the most common organism isolated from patients with asymptomatic bacteriuria (Geerlings *et al*; 2000). These bacteria may be resistant to multiple antibiotics, and thus, they pose a serious threat to the safety and proper functioning of the patient. Untreated asymptomatic bacteriuria predisposes an individual to recurrent UTI, which may cause renal diseases. This study was aimed at quantifying the proportion of women at Michael Okpara University of Agriculture, Umudike who had asymptomatic bacteriuria.

MATERIALS AND METHODS

MATERIALS

Urine samples were collected from a total of 80 female students of the Michael Okpara University of Agriculture Umudike between the ages of 19 to 25. Students who had taken antibiotic two weeks before or were currently in antibiotics were excluded from the study.

COLLECTION OF SAMPLE

Midstream urine samples were collected from female student that visited the university clinic. The samples were collected inside sterile universal bottles and were examined immediately.

STERILIZATION OF MEDIA AND MATERIALS

The media used were Nutrient Agar, MacConkey Agar and Blood Agar. All glassware were washed and rinsed then allowed to dry. The glassware were wrapped in aluminum foil and sterilized in an oven at 160^0C for 1 hour. Media were prepared according to the manufacturer's specification and sterilized by autoclaving at 121^0C for 15minutes.

MICROSCOPY

A portion of urine samples were poured into plastic test tubes and centrifuged at 2000rpm/s for 10minutes.The supernatural was decanted and used. Using a drop Pipette, two drops were put on a microscope slide with cover slip and viewed under X40 objective of a light microscope (Wogu and Ogbebor, 2011). Urinalysis was carried out using a urine dipstick to evaluate the presence of nitrite reducing bacteria.

ISOLATION OF BACTERIAL SPECIES

Each of the urine samples were subjected to culturing using the spread plate technique. This was carried out as described by Cheesbrough (2002). A glass rod dipped in ethanol and flamed was used to spread each sample on Nutrient agar in Petri dishes. The contents were allowed to gel and the plates were then incubated at 37^0C for 24hours. Bacterial colonies appearing on the plates after the incubation period were enumerated to determine urine samples with significant bacteriuria (> 10^5cfu/ml). An aliquot of the urine sample was also transferred onto MacConkey and Blood agar for the isolation of the bacteria present in the urine. After incubation, plates with growth were selected; the colonies were isolated using inoculating loop and subsequently sub cultured on agar slants for use in further tests.

IDENTIFICATION OF ISOLATES

The methods used in the identification and characterization of isolated bacteria include Gram stain, followed by microscopic examination and other biochemical tests (including Indole, Methyl red, Voges-Proskauer, Urease, Catalase, Hydrogen Sulfide and Coagulase) according to Cheesbrough (2002).

SUBCULTURE AND PURIFICATION

After incubation of the plates with the different media, discrete colonies from the plates were picked with a flamed wireloop and sub cultured onto newly prepared nutrient agar plate. The plates were incubated at 37^OC for 24-48 hours. The purified colonies were transferred into slants and stored properly for further characterization.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antibiotic sensitivity test was performed by disc diffusion technique using commercially available antibiotic impregnated discs on Mueller-Hinton agar. Each of the isolated organisms were taken from the slant and cultured onto nutrient agar plates for 24 hours. Well isolated colonies from the 24hour nutrient agar culture were transferred into sterile bottle containing normal saline and swabbed with a sterile swab stick onto the surface of Meuller-Hinton agar. Multi discs containing Ampicillin, Tetracycline, Augmentin, Ofloxacin, Cotrimoxazole, Gentamicin, Ciprofloxacin and Chloramphenicol were placed on the inoculated agar surface, allowed to stand for few minutes and then incubated at 37^OC for 24 hours. The zone(s) of inhibition produced after overnight incubation were measured to the nearest millimeter (mm) and recorded.

RESULTS

A total of eighty (80) female student urine samples were examined and analyzed for asymptomatic bacteriuria. The total number of positive cultures (with significant growth) 39 out of 80 samples analyzed representing 48.7% is presented in table 4 while 51.3% was with insignificant growth. Culture plates with bacteria count greater than or equal to 10^5cfu/ml were considered significant and thus indicative of asymptomatic bacteriuria.

The bacterial isolates and their percentage prevalence of the organisms is as shown in table 3. *Escherichia coli* was found to be the most prevalent (22.5%), followed by *Staphylococcus aureus* (12.5%) and *Klebsiella spp* (5.00%). The least prevalent organisms were *Proteus spp and Streptococcus spp* with 5.00% and 3.75% respectively.

The bacterial isolates were identified based on colony morphological characteristic, Gram stain reaction and several biochemical tests. The microscopic examination revealed that of the 80 samples examined, pus cells were found in 47 (58.7%) and Yeast cells 7 (8.75%) as in table 2. The results of the urine dipstick analysis are as presented in table 1.

The results of susceptibility to antimicrobial agents among the bacterial isolates analyzed are provided in table 5. Ampicillin and tetracycline had the highest number of resistant isolates while Ciprofloxacin, Ofloxacin and Gentamicin showed the least (i.e.) most of the isolates were found to be susceptible to Ciprofloxacin, Ofloxacin and Gentamicin (table 5).

TABLE 1: Urinalysis for Nitrite and Leucocyte Esterase.

Number of Urine Specimen Screened	Number (%) Positive for Nitrite	Number (%) Positive for LE
80	26 (32.5)	20 (25.0)
LE = Leucocyte Esterase		

TABLE 2: Microscopic Examination

Isolates	Number of Positive Samples (%)
Pus cells	47 (58.7)
Yeast cells	7 (8.75)

TABLE 3: Frequency of Isolation of Organisms

Isolates	Number of Positive Samples (%)
Escherichia coli	18 (22.5)
Staphylococcus aureus	10 (12.5)
Klebsiella spp	4 (5.00)

Proteus spp	4 (5.00)
Streptococcus spp	3 (3.75)
Total	39 (48.7)

TABLE 4: Levels of Bacteriuria (Significant Growth).

Number of Occurrence		Percentage Occurrence
Samples with significant growth	39	48.7
Samples with insignificant growth	41	51.3
Total	80	100

TABLE 5: Antimicrobial Susceptibility Rates of Bacterial Isolates against Antimicrobial Agents

EC = *Escherichia coli;* SA = *Staphylococcus aureus;* K. spp = *Klebsiella* species*;* P. spp = *Proteus* species*;* Strepp. Spp. = *Streptococcus* species

Agent	SA (N=10)	EC (N=18)	K.spp (N= 4)	P.spp (N=4)	Strep.spp (N=3)
Ampicillin	0 (0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Chloramphenicol	2(20.0%)	5(27.8%)	1(25.0%)	0(0.0%)	0(0.0%)
Ciprofloxacin	6(60.0%)	9(50.0%)	4(100.0%)	4(100.0%)	2(66.7%)
Tetracycline	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Gentamicin	7(70.0%)	12(66.7%)	3(75.0%)	3(75.0%)	3(100.0%)
Cotrimoxazole	4(40.0%)	0(0.0%)	0(0.0%)	2(50.0%)	0(0.0%)
Ofloxacin	10(100.0%)	16(88.9%)	4(100.0%)	4(100.0%)	3(100.0%)
Augmentin	2(20.0%)	5(27.8%)	0 (0.0%)	1(25.0%)	0(0.0%)

DISCUSSION

Asymptomatic bacteriuria is common, with varying prevalence by age, sexual activity and the presence of genitourinary abnormalities. Lyamuya *et al*., 2010, reported that the prevalence of asymptomatic bacteriuria is 2-3 times higher among women with diabetes mellitus compared to women without it. Alterations in vaginal micro flora due to consumption of unprescribed drugs also play a critical role in encouraging vaginal colonization with pathogens and this can lead to urinary tract infection (Hooton *et al*., 1995).

The pathogens involved in urinary tract infection are almost always predictable with *Escherichia coli* being the most common among others. The occurrence of asymptomatic bacteriuria (Table 4) in this study was 48.7%. This result was comparable to the 47.5% reported by Okonko *et al* (2000). The decreased percentage occurrence could be attributed to extensive healthcare talks given regularly in schools and public awareness campaigns. Kunar *et al* (2002) found the organisms most frequently isolated to include species of Enterobacteriaceae especially *E. coli* and other Gram negative bacteria. The findings of this study also are consistent with the study conducted by Kunar *et al* (2002). The most common

isolated organism in this study was found to be *Escherichia coli* and was responsible for 22.5% of the cases. It was followed by *Staphylococcus aureus* (12.5%); *Klebsiella* species (5.00%), *Proteus* species (5.00%) and *Streptococcus* species accounted for 3.75%. The findings of this study revealed that the important infecting organisms were the commensals of perianals and vaginal regions. This therefore calls for increase in personal hygiene practices. Aiyegoro *et al* (2007) also reported that *E. coli* was the most commonly isolated pathogen in significant bacteriuria.

The findings of this study are consistent with the hypothesis that asymptomatic bacteriuria is a frequent occurrence that results when urinary tract pathogens, particularly *E. coli* enter the bladder without causing symptoms. The pathogens are however, usually eliminated by host defense factors (Hooton *et al.*, 2000), but they may persist for a short or rarely a long time (because of their adhesion properly) or result in symptomatic urinary tract infection.

The predominance/occurrence of *E. coli* in this study as well as other studies could be as a result of poor genitalia hygienic practices by women who find it difficult to wash up their genitalia after passing urine.

The Nitrite pad of the urine strip provides a rapid screening test for the presence of bacteria that are often responsible for urinary tract infections. The percentages recorded in this study did not however prevent us from proceeding with culturing to avoid false positive results. This could occur when the urine samples are left at room temperature for an extended period of time, allowing bacterial contaminants to multiply and produce more nitrite. Negative results may also occur in the samples if the urine specimen didn't stay long enough in the bladder for bacteria to reduce enough quantity of nitrates to nitrite.

Asymptomatic bacteriuria and UTI are also associated with females due to anatomical factors (for example, short length of the urethra hence lesser distance of bacteria ascending up the urinary tract); hormonal changes affecting the adherence of bacteria to the mucosa as well urethral trauma during sexual intercourse.

The result of antibiotic sensitivity (Table 5) showed that Ciprofloxacin, Ofloxacin and Gentamicin were effective against most of the isolates with Ofloxacin achieving a sensitivity of 88.9% against *E. coli* being the most predominant isolate. The sensitivities recorded by

these antibiotics are likely due to their broad spectrum of activity on bacteria. The result also showed that there is a rapid decline in Ampicillin and Tetracycline sensitivity.

The higher resistances observed in this study to Ampicillin and Tetracycline could be attributed to their free access, low cost of purchase, buying drugs without prescription and abuse. Other studies by Inabo and Obanibi (2005) reported similar observations. The widespread and inappropriate use of antibiotics is seen as a significant contributing factor to the spread of bacterial resistance and the development of resistance to antimicrobial agents.

CONCLUSION

Urinary tract infection (UTIs) may be symptomatic and asymptomatic in most cases. Since public enlightenment is yielding result, more of such programmes should be encouraged in schools, the media and markets to further reduce the percentage recorded in this study.

The incidence rate of 48.7% recorded in this study shows that there is a considerable decline in the rate of asymptomatic bacteriuria that predisposes people to UTI. Further studies needs to be carried out to discover more antibiotic that will be sensitive to the most common organism (*E. coli*) associated with urinary tract infection**.**

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