Asymptomatic urinary tract infection amongst some Students of Michael Okpara University of agriculture, Umudike

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This work was carried out to determine Asymptomatic Urinary Tract Infection amongst some students of Michael Okpara University of Agriculture, Umudike and the sensitivity pattern of the isolates from urine. Using aseptic technique, midstream urine were collected from sixty (60) students, urinalysis was carried out on the urine samples and was then cultured on CLED and MacConkey agar using pour plate method. Growth was observed in 26 (87%) of the sample while there was no growth in 4 (13%) of the sample. Out of the 26 (87%) samples with growth, 14 (47%) had significant bacteria growth while 12 (40%) had no significant growth. Incidence of asymptomatic bacteriuria was higher in females 8 (57%) than males 6 (38%). The organisms isolated were Escherichia coli, Klebsiella species, Staphylococcus saprophyticus, Staphylococcus aureus, Enterococcus faecalis, Proteus species, and Pseudomonas aeruginosa. All the Gram positive isolates were sensitive to Gentamycin and all resistance to Cefuroxime, Ceftazidime, Ceftriaxone, Cloxacillin. The Gram negative isolates were mostly sensitive to Nitrofurantoin, Gentamycin and Ofloxacin. Therefore, these drugs could be considered as the first line of drug for the treatment of asymptomatic urinary tract infection.

Keywords: Asymptomatic, urinary tract infection, students, assessment, rate.

INTRODUCTION

Urinary Tract Infection mostly occurs in patients with anatomically and functionally normal urinary tract and usually results from spontaneous ascent of bacteria from the urethra to the bladder. Occasionally, the bacteria progresses to the kidney and blood stream. However, since asymptomatic colonization of the urinary tract can occur, other features such as the presence of inflammatory markers or follow up cultures are needed to correctly diagnose a person with urinary tract infection. Urinary Tract Infection (UTI) represents 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 one of the most common bacterial infection in human body (Tambekar et al, 2006). Urine is generally considered to be sterile and is believed to be Germ Free, any source of possible infection occurs through Urethra which initiates the incidence of the infection. Urinary Tract Infection is caused by the presence of bacteria in urine, although fungi and viruses could be involved (Siiri et al, 2009). The predominant pathogen responsible for Urinary Tract Infection is Escherichia coli which constitute up to 75 – 90% and is followed by Staphylococcus saprophyticus which accounts for 5– 15% of Urinary Tract Infection (Michael et al, 2007). In addition to the above mentioned bacterial species, Klebsiella, Proteus, Pseudomonas and Enterobacter are associated with UTI.

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Pathogens like *Escherichia coli* and *Staphylococcus saprophyticus* are associated with population acquired acute uncomplicated infection where as *Klebsiella, Enterococcus, Proteus* species, *Enterobacter* are known to confer uncomplicated cystitis and phylonephritis that are sporadic (Mars, 2002). Asymptomatic UTI 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 a human host. However, when such pathogens stay within the urinary system for a long time, symptomatic urinary tract infection results (Nicolle, 2003). Asymptomatic bacteriuria is defined by the presence of at least 10^5 colony forming units (CFU) of organisms per milliliter in cultures of urine specimens in the absence of symptoms of infection referable to the Urinary Tract (Smith, 1994). Asymptomatic bacteriuria occurred reliably more frequently in females as compared to males and it is a major criterion of urinary tract infection (Nurullaev 2004). Therefore, it is necessary to investigate the incidence of Asymptomatic bacteriuria in both apparently healthy male and female undergraduate students of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

**MATERIALS AND METHODS**

**Study Site**

Michael Okpara University of Agriculture, which is located along Ikom-Ekpeno road, Umudike, in Ikwuano Local Government Area, Abia State, Nigeria.

**Sample Collection**

The participated students were instructed to wash their external genital with mild toilet soap and rinse thoroughly with clean water. Early morning mid-stream urine samples of about 10-15ml were collected using a sterile universal bottle with air tight screw cap tops. Each urine sample bottle was labeled with a name, number, sex, department, and time of collection, etc. Each of the students was instructed on the mode of collection of the mid-stream urine and also adequately educated on precautions to prevent contamination of the specimen.

**Inclusion Criteria**

All apparently healthy students who indicated the absence of symptoms from questionnaires issued where included in the study.

**Exclusion Criteria**

Students who had taken antibiotics two weeks before or were currently on antibiotic therapy, or with any symptoms suggestive of UTI or pregnancy as indicated in the questionnaire were excluded from the study.

**PROCESSING OF SAMPLES**

**Urinalysis**

This is used as a pretest to check for Asymptomatic Urinary Tract Infection or symptomatic urinary tract infection. A medi-test Combi-9 impregnated with test strip for urine parameters was dipped into urine samples in a test tube, the reactions were examined visually immediately after allowing excess urine to drip off comparing the colours produced against the colour shade used for charting and estimating the reactions. The parameters tested were Nitrite, Proteins H, Ketones, Glucose, Bilirubin, Urobilinogen, Blood. An Asymptomatic UTI indicates negative to Nitrite, protein and few pus cells (leucocytes) while a symptomatic UTI indicates positive to Nitrite, protein and many pus cells (leucocytes).

**Urine Microscopy**

This is used as a pretest to check for UTI, a well mixed urine sample was aseptically transferred into a test tube and centrifuged at 500-1000g for about 5minutes. The supernatant fluid was discarded by completely inverting the tube, the sediment was remixed by tapping the bottom of the tube, then a drop of the well mixed sediment was transferred to the glass slide and covered with a cover slip. It was then examined under the microscope using x10 objective to focus and x40 objective to view. The leucocytes (pus cell) counts are usually reported as: Few-up to 10/HPF (High Power Field), 11-40/HPF (moderate), and more than 40 WBC/HPF (many). Above 40 indicates Urinary Tract Infections.

**Culturing of Urine Samples**

Each of the Urine samples was observed macroscopically to ascertain if the urine was clear or cloudy (turbid). Each urine sample was shaken properly to ensured homogeneity. Ten fold serial dilutions were made of each specimen and 1ml of the appropriate dilutions was used as inoculums using the pour plate method, culturing in cysteine – lactose electrolyte deficient (CLED) agar and Mac Conkey agar. The cultured plates were incubated aerobically at 37°C for 24hours (Cheesbrough, 2000). The plates were examined macroscopically after 24 hours, the bacterial colonies were counted and multiplied by 100 to given an estimate of the number of bacteria present per millimeter of urine. A significant bacteria count was taken as any count equal or in excess of 10,000 CFU/ml. Plates with growth at 10^5 and above were indicated as significant bacteriuria while plates that did not show growth at 10^5
Table 1. Age and gender association with UTI

<table>
<thead>
<tr>
<th>AGE</th>
<th>Gender</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>16 – 20</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>21-25</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>≥ 26</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>16</td>
<td>30</td>
</tr>
</tbody>
</table>

Antimicrobial Susceptibility Testing

Antimicrobial sensitivity was tested for each isolated organisms to determine the antibiotic susceptibility pattern of the different isolates. The agar diffusion disc techniques described by Bauer et al (1996) was used. A colony of pure isolate was streaked on sterile Mueller Hinton agar plates aseptically using sterile inoculating wire loop. The appropriate multi disc containing minimum inhibitory concentrations (MIC) of Ciprofloxacin (5μg), Nitrofurantoin (300μg), Ceftriaxone (30μg), Gentamycin (10μg), Cefuroxime (30μg), Ofloxacine (5μg), Cefixime (5μg), Cefazidime (30μg), Ampicillin/Clavulanate (30μg), Erythromycin (5μg) and Cloxacillin (5μg) was aseptically placed firmly onto the surface of the dried plates using a sterile forceps. After 30mins of applying the disc, the plates were kept at room temperature to allow diffusion of the different antibiotics from the disc into the medium. The plates were then inverted and incubated at 37°C for 24hours. The clear zone that developed around each disc was measured as the zones of inhibition from underneath each plate with the aid of a ruler in millimeter (mm). The reaction of the test organism to each antibiotics was reported, sensitivity and resistance patterns were recorded in accordance with the European Committee on antimicrobial susceptibility testing (EUCAST) Version 6.0 recommendations.

Results

Urine samples from sixty (60) students of Michael Okpara University of Agriculture, Umudike within the age range of 16 - ≥ 26 years were collected and examined for the presence of bacteria using a semi-quantitative method. The colour of the urine samples ranged from pale yellow to light yellow, some urine samples were clear and some turbid in appearance. From the urinalysis leucocytes (pus cells) were observed in 29 samples, 26 samples were positive to Nitrite and 13 samples were positive to protein. Detection of Nitrite, leucocyte and protein is a rapid method for detecting Asymptomatic Urinary Tract Infection. The urine samples had the normal pH range of 4.0 – 8.0. Table 1 shows the Age and Gender association with UTI. Out of the 60 urine samples examine, 50% of the positive cases were observed from those within the age range of 21 - 25, this was followed by those within the age range of 16 – 20 (30%) while the list was from among those within the age range of ≥ 26 (20%).

The incidence of Bacteriuria among the students is shown in Table 2. Out of the 30 urine samples cultured (those that were negative from the preliminary test–urinalysis/microscopy), 14 samples (47%) showed significant bacteria growth, and 16 samples (53%) showed no significant bacteria growth.

The characterization and identification test carried out reveals the bacterial isolates associated with UTI to belong to the genera *Escherichia coli*, *Klebsiella* species, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus* species and *Pseudomonas aeruginosa*.

Table 3 shows the frequency of occurrence of the bacteria isolates from the urine samples with significant bacteriuria. *Escherichia coli* had the highest frequency 10(33%) followed by *Klebsiella* species 5(17%), *Staphylococcus saprophyticus* and *Staphylococcus aureus* had 4(13%) frequency of occurrence respectively. The list frequency was observed with *Proteus* species and *Pseudomonas aeruginosa* 2(7%) respectively.

Table 4 (a) and 4(b) depicts the Antibiotic susceptibility pattern of the isolates. Majority of the Gram positive bacteria were sensitive to Gentamycin, Erythromycin and Ofloxacine while some others were resistant to the
antibiotics. Majority of the Gram negative bacteria were sensitive to Gentamycin, Ofloxacin and Nitrofurantoin while some were resistant to the antibiotics.

### DISCUSSION

This work was carried out to determine the presence of Asymptomatic Urinary Tract Infection among sixty (60) students of Michael Okpara University of Agriculture, Umudike. 30 urine samples were positive to UTI after a pretest of urinalysis and urine-microscopy. From the urinalysis, 27 samples were positive to Nitrite, 12 samples were positive to protein and 29 leucocytes (pus cells) were observed. Detection of these 3 parameters is rapid method for detecting Asymptomatic UTI. The report is in line with (Bent, 2002) that dipstick urinalysis in practice may provide useful information for the diagnosis of UTI. The presence of nitrite has predictive value of UTI, since nitrite are formed as a metabolic product from bactecria that break down nitrate to nitrite e.g. E. coli, Proteus spp, Klebsiella spp etc. If the dipstick is positive to either nitrate or leucocyte this increases the probability of UTI to about 80% while dipstick negative in both nitrate and leucocyte reduces the probability of UTI to about 20% (Bent, 2002).

For this study, the significant Asymptomatic UTI is $10^5$ CFU/ml (Cheesbrough, 2000). Asymptomatic bacteriuria was observed in 47% of the population. The higher incidence of Asymptomatic UTI rate of female (57%) compared to that of male (38%) is in agreement with Prescott et al (2004) who reported that bacteriuria is more common in females and is often asymptomatic with frequent reoccurrence. Higher cases of Asymptomatic UTI in female than male has also been observed by other workers (Frank-Peterside and Oguike, 2006), the higher incidence in female than male could be due to the shorter length of the Urethra in females and its closeness to the
Asymptomatic urinary tract infection amongst some Students of Michael Okpara University of Agriculture, Umudike

Table 4 (a). Susceptibility pattern of the isolates using gram positive antibiotic disc

<table>
<thead>
<tr>
<th>Group Of bacteria</th>
<th>Isolate</th>
<th>No. Isolated</th>
<th>Caz</th>
<th>Crx</th>
<th>Gen</th>
<th>Ctr</th>
<th>Ery</th>
<th>Cxc</th>
<th>Off</th>
<th>Aug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram positive</td>
<td>Staphylococcus saprophyticus</td>
<td>4</td>
<td>4R</td>
<td>4R</td>
<td>4S</td>
<td>4R</td>
<td>4S</td>
<td>4R</td>
<td>4R</td>
<td>1S3R</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>4R</td>
<td>4R</td>
<td>4S</td>
<td>4R</td>
<td>4S</td>
<td>4R</td>
<td>2S2R</td>
<td>4R</td>
</tr>
<tr>
<td></td>
<td>Enterococcus faecalis</td>
<td>3</td>
<td>3R</td>
<td>3R</td>
<td>3S</td>
<td>3R</td>
<td>1S2R</td>
<td>3R</td>
<td>3S</td>
<td>2S1R</td>
</tr>
</tbody>
</table>

KEY: S = Sensitive, R = Resistance
Caz = Ceftazidime; Crx = Cefuroxime; Gen = Gentamycin; Ctr = Ceftriaxone; Ery = Erythromycin; Cxc = Cloxacillin; Off = Ofloxacin; Aug = Augmentin.

Table 4 (b). Susceptibility pattern of the isolates using gram negative antibiotic disc.

<table>
<thead>
<tr>
<th>Group Of bacteria</th>
<th>Isolate</th>
<th>No. Isolated</th>
<th>Caz</th>
<th>Crx</th>
<th>Gen</th>
<th>Czx</th>
<th>Ofl</th>
<th>Amx/C</th>
<th>Nit</th>
<th>Cpr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Negative</td>
<td>Escherichia coli</td>
<td>10</td>
<td>10R</td>
<td>9S1R</td>
<td>10S</td>
<td>10R</td>
<td>10S</td>
<td>8S2R</td>
<td>10R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella species</td>
<td>5</td>
<td>5R</td>
<td>5S</td>
<td>5S</td>
<td>5R</td>
<td>3S2R</td>
<td>5R</td>
<td>5S</td>
<td>5S</td>
</tr>
<tr>
<td></td>
<td>Proteus species</td>
<td>2</td>
<td>2R</td>
<td>2R</td>
<td>2R</td>
<td>2R</td>
<td>2S</td>
<td>2R</td>
<td>2S</td>
<td>2S</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>2R</td>
<td>2R</td>
<td>2S</td>
<td>2R</td>
<td>1S1R</td>
<td>2R</td>
<td>2S</td>
<td>1S1R</td>
</tr>
</tbody>
</table>

KEY: S = Sensitive, R = Resistance
Caz = Ceftazidime; Crx = Cefuroxime; Gen = Gentamycin; Czx = Cefixime; Ofl = Ofloxacin; Amx/C = Amoxicillin/Clavulanate; Nit = Nitrofurantoin; Cpr = Ciprofloxacin.

external passage that facilitates the entrance of microorganism into the body. Also the high rate may be due to the increase in female with poor hygiene practice and also indiscriminate sexual behavior among the female students.

Seven (7) bacterial species, *Escherichia coli*, *Klebsiella* species, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus* species, and *Pseudomonas aeruginosa* were isolated from this study. These species have also been isolated in similar studies by different investigators of asymptomatic UTI (Roos *et al*, 2006).

The highest frequent occurring organism isolated from this study was *Escherichia coli* (33%). The presence of *Escherichia coli* as the commonest etiological agent of UTI has also been reported in previous studies by (De Francesco *et al*, 2001). The second most common organism isolated in this study was *Klebsiella* species with (17%), followed by *Staphylococcus saprophyticus* and *Staphylococcus aureus* (13%) respectively. This is usually found in infection among sexually active young women and can cause UTI in males of all ages (Cheesbrough, 2004). The least frequency was observed with *Proteus* species and *Pseudomonas aeruginosa* (7%).
respectively. Their presence in the urine sample should however not be overlooked because they have been reported as agents of urinary tract infection (Cheesbrough, 2002). This work has showed that some bacterial has showed that some bacterial isolates are more susceptible to treatment with certain antibiotics than others. Majority of the Gram positive bacteria were sensitive to Gentamycin, Erythromycin and Ofloxacin while some Gram negative bacteria were sensitive to Nitrofurantoin, Ofloxacin and Gentamycin. These drugs could be considered as among the first line of drugs for treating cases of urinary tract infection.

The least resistance of the bacterial isolates to antimicrobial agents was observed to be Caftazidime, Cefuroxime, Ceftriaxone, Cloxacillin, Amoxicillin/Clavulanate and Cefixime as seen from this study. The factors contributing to those resistances may be due to indiscriminate abuse of antibiotic by students. Other factors may include poor quality of drugs, poor storage and expose drug (Okeke et al, 1999) etc. the reduction of antibiotic prescription and dispensation have been associated with reduced antibiotic resistance (Schiemann et al, 2010).

CONCLUSION


REFERENCES


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