A total of 118 urine samples taken for Staphylococcus, sexual habits especially of underwear's. O; Gould other bacteria pathogens frequently isolated and female patients of all age groups with suspected cases of Collection of samples clinic. The study population was drawn from a total of 118 patients attending Specialist Hospital Benin City, Edo State, Nigeria. A total of 118 urine samples taken from both male and female patients across different age group were analyzed. Five bacteria genera were isolated with Staphylococcus aureus being the most prevalent (28%), followed by Escherichia coli (18.6%), Klebsiella pneumoniae (13.6%), Proteus mirabilis (10.2%) and Pseudomonas aeruginosa (6.8%) while no growth was recorded in 22.9% of samples. Uropathogens were higher in females (60.4%) compared to males (39.6%). A record of 41.6% cases were observed among patients between the ages of 21 - 30 years and the majority were female (47.3%). The antibiogram pattern of all isolates showed sensitivity for nitrofurantoin. This result suggests the use of nitrofuratoin for empirical treatment of UTI and that bacteria resistance of uropathogens requires periodic drugs resistance surveillance.

Keywords: Antibiotics, gentamicin, Staphylococcus aureus, sensitivity, uropathogen.

Introduction
The urinary tract helps the body to get rid of toxic waste substance especially urea by passing them out through openings on the genital organs (Farooqui, et. al., 1989). A number of different microorganisms have been known to cause urinary tract infections; these include those of the normal flora of the skin, genital areas, anus and those from exogenous sources that maybe contacted through bad sanitary habits especially of underwear’s (Nicolle, 2008). Some of the risk factors of UTI include gender, sexual activity, immune system disorder, urinary tract anatomical malformations, disruption of normal flora of the genital area with antiseptics and antibiotics, urinary catheter and instrumentation (Foxman, 2003; Hooton, 1990; Nicolle, 2008; Gould et. al., 2010). About 95% of urinary tract infection occurs when bacteria ascend the urethra to the bladder or ascend the ureter to the Kidney (Farooqui et. al., 1989). Urinary tract infection occurs much more frequently in female than male due to the proximity of the urethra to the anus. Approximately 50% of all women will have at least one UTI in her life time with many women having several infections through their life time (Foxman, 2003).

Gram negative bacteria have been found most frequently in UTI cases by several authors with E. coli and Klebsiella pneumoniae being the most predominant organisms (Obiogbolu, 2004). Other bacteria pathogens frequently isolated include S. aureus, S. epidermidis and Streptococcus faecalis (Eghafona et. al., 1998). The degree and prevalence of occurrence of one or two of these organisms over others depends on the environment (Omonigho et. al., 2001).

UTI may be classified into urethritis, cystitis and pyelonephritis depending on the site of infection. The commonest mode of infection is the ascending route, through which organisms of the bowels flora contaminates the urethra, ascend to the bladder and migrates to the kidney or prostrate (Stamm, 1988).

Treatment regimen of UTI has been based largely on antibiotics and occasional surgery which is done to drain abscesses, correct underlying structural abnormalities or relieve obstructions. This study was aimed at evaluating the frequency of the most causative agents of UTI and the antimicrobial susceptibility pattern of the pathogenic isolates of the urinary tract.

Materials and Methods
Study population
The study population was drawn from outpatients attending Specialist Hospital, Benin City, Edo State, Nigeria. Urine samples were collected from a total of 118 patients of all age group between October 2012 - December 2012. In the collection of urine from patients, the following exclusion criteria were used: Patients who have been on antibiotics for at least three days were excluded and patients who took large quantities of fluids less than an hour before coming to clinic.

Collection of samples
A total of 118 clean voided mid-stream urine samples were collected in sterile universal containers from male and female patients of all age groups with suspected cases of UTI. At point of collection, samples were labeled appropriately and immediately investigated for microscopy, culture and sensitivity analysis.
Sterilization of media and materials

The media used were Blood Agar (BA) and MacConkey agar (MCA). All glassware was washed with detergent and rinsed with water, then allowed to dry. The glassware were later wrapped in aluminum foil and sterilized in a hot air oven at 160°C for 3hrs. Media were sterilized by autoclaving at 121°C for 15min.

Microscopy

The urine samples were mixed and aliquots centrifuged at 5000 rpm for 5min. The deposits were examined using both x10 and x40 objectives. Samples with ≥10 white blood cells/mm³ were regarded as pyuric (Smith et al., 2003).

Culture

A calibrated sterile platinum wire loop for the semi-quantitative method was used for the plating and it has a 4.0 mm diameter designed to deliver 0.01 ml. A loopful of the well mixed urine sample was inoculated into duplicate plates of Blood and MacConkey agar. All plates were then incubated at 37°C aerobically for 24hrs. The plates were then examined macroscopically and microscopically for bacterial growth. The bacterial colonies were counted and multiplied by 100 to give an estimate of the number of bacteria present per milliliter of urine. A significant bacterial count was taken as any count equal to or in excess of 10,000 cfu /ml (Stamm et al., 1982; Stark and Maki, 1984).

Bacterial identification

The identification and characterization of bacteria isolates were done on the basis of cultural, colonial and morphological appearance on culture media and by conventional biochemical test with reference to Cheesbrough, 2000; Prescott et al., 1999.

Antibiotic sensitivity test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar diffusion method as described by Baur et al., (1996). Nutrient agar plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the nutrient agar plates and allowed to stand for 1hour. The plates were then incubated at 37°C for 24 hours. The diameter of the zone of inhibition produced by each antibiotic disc was measured using a meter rule and the result was interpreted as sensitive if above 14mm or resistant if below 14mm.

Results

Table 1 shows the bacteria isolates and its distribution in percentage from urine samples of patients with cases of UTI. Staphylococcus aureus was found to be the most prevalent organism 33(28.0%) while Pseudomonas aeruginosa 8(6.8%) was least prevalent.

The relationship between sex and age of patients with UTI is shown in table 2. The female presents 60.4% while the males accounted for 39.6% of the bacterial isolates and the age group between 21-30 years had the highest number of isolates in both sexes.

Table 3 shows the antibiogram pattern of the bacteria isolates to ten commonly used antibiotics. S. aureus, K. pneumoniae and P. aeruginosa were resistant to seven of the ten antibiotics. All bacteria isolates were sensitive to nitrofurantoin while they were all resistant to cefotaxine, ofloxacin, ocefix, cloxacillin and cotrimoxazole.

Table 1: Distribution in percentage of bacteria isolates from urine samples of patients with cases of UTI.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>K. pneumoniae</th>
<th>P. mirabilis</th>
<th>P. aeruginosa</th>
<th>No growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 10</td>
<td>1(4.5)</td>
<td>1(3.0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>9(33.3)</td>
</tr>
<tr>
<td>11 – 20</td>
<td>6(27.2)</td>
<td>10(30.3)</td>
<td>8(50)</td>
<td>3(25.0)</td>
<td>1(12.5)</td>
<td>5(18.5)</td>
</tr>
<tr>
<td>21 – 30</td>
<td>8(36.3)</td>
<td>16(48.4)</td>
<td>53(1.2)</td>
<td>6(50.0)</td>
<td>3(37.5)</td>
<td>3(11.1)</td>
</tr>
<tr>
<td>31 – 40</td>
<td>4(18.1)</td>
<td>3(9.0)</td>
<td>2(12.5)</td>
<td>1(8.3)</td>
<td>1(12.5)</td>
<td>2(7.4)</td>
</tr>
<tr>
<td>41 – 50</td>
<td>2(9.0)</td>
<td>1(3.0)</td>
<td>1(6.2)</td>
<td>2(16.6)</td>
<td>1(12.5)</td>
<td>2(7.4)</td>
</tr>
<tr>
<td>50±</td>
<td>1(4.5)</td>
<td>2(6.0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>2(25.0)</td>
<td>7(25.9)</td>
</tr>
<tr>
<td>Total</td>
<td>22(18.6)</td>
<td>33(28.0)</td>
<td>16(13.6)</td>
<td>12(10.2)</td>
<td>8(6.8)</td>
<td>27(22.9)</td>
</tr>
</tbody>
</table>

Table 2: Percentage distribution of isolates from urine samples of patients screened in relation to age and gender.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males %</th>
<th>Female %</th>
<th>Totals %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>1(2.8)</td>
<td>1(1.8)</td>
<td>2(2.2)</td>
</tr>
<tr>
<td>11-20</td>
<td>11(30.6)</td>
<td>17(30.9)</td>
<td>28(30.8)</td>
</tr>
<tr>
<td>21-30</td>
<td>12(33.3)</td>
<td>26(47.3)</td>
<td>38(41.6)</td>
</tr>
<tr>
<td>31-40</td>
<td>4(11.1)</td>
<td>7(12.7)</td>
<td>11(12.1)</td>
</tr>
<tr>
<td>41 – 50</td>
<td>5(13.9)</td>
<td>2(3.6)</td>
<td>7(7.7)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>3(8.3)</td>
<td>2(3.6)</td>
<td>5(5.5)</td>
</tr>
<tr>
<td>Total</td>
<td>36(90.6)</td>
<td>55(60.4)</td>
<td>91(100)</td>
</tr>
</tbody>
</table>

Table 3: Antibiogram pattern of the bacteria isolates.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Aug</th>
<th>Gen</th>
<th>Ctx</th>
<th>Nit</th>
<th>Ofl</th>
<th>Ocf</th>
<th>Cxc</th>
<th>Cro</th>
<th>Ox</th>
<th>cot</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>
S = Sensitive, R = Resistant, AUG = Augmentin, GEN = Gentamicin, CFX = Cefotaxime, NIT = Nitrofurantoin, OFL = Ofloxacin, OCF = Ocefix, CXC = Cloxacillin, CRO = Rocephin , OX = Askarivid, COT = Cotrimoxazole

Discussion

This study showed the prevalence of bacteria as possible etiological agents of UTI cases. Five bacteria species were isolated and they include E. coli, S. aureus, K. pneumoniae, P. mirabilis and P. aeruginosa and these finding were earlier reported by Akortha and Ibadin (2008); Obiogbolu et. al, 2009; Ojo and Anibijuwn (2010). *Staphylococcus aureus* was found to be the most prevalent uropathogen with 28% of all isolates. This finding agrees with the reports of Sulé (1991); Akerele and Ahonkhai (2000) that *S. aureus* is the most frequent organisms isolated from urine cultures and it is the leading etiological agent in urinary tract infection in this environment of study. The high incidence of *S. aureus* could be as a result of its ability to overcome body defense mechanisms and resistance to antibiotics. This work however is in contrast to the work of Okonofua, 1995; Fibie et. al, 2001 and Omonigho et. al., 2001 who has independently reported *E. coli* and Klebsiella sp. as the most predominant organisms in UTI cases.

*E. coli* was next in the percentage prevalence with 18.6% while *P. aeruginosa* was least with 6.8%. From the total number of urine sample investigated, 22.9% of the sample yielded no significant growth after 48 hours incubation despite the significant level of pus cells, epithelial cells and red blood cells present in the microscopy sediments of the urine samples. This could be attributed to high and indiscriminate use of antibiotics by patients when urinary disorders are noticed.

This study also revealed a higher incident of UTI in female than male (Table 2) which could be attributed to the proximity between the genital tracts, urethra and anus which perhaps facilitate auto transmission as earlier suggested by Audu and Kudi, (2004). The moist environment of the female perineum could also favour microbial growth and bladder contamination as reported by Ebie et. al, (2001). Furthermore, the highest incidence of UTI was recorded between the ages of 21 – 30 with 41.6% with women in this group accounting for 47.3%. This was followed by an incidence of 30.8% among the ages of 11 – 20 years with the females accounting for 30.9% (Table 2). This study therefore supported the report Ebie et. al,(2001) that there is high rate of bacterial infection among sexually active women of child bearing age. This is because the two age groups with the highest incidence of UTI are considered to be the sexually active, child bearing and adolescent age groups. The lowest incidence for both male (2.8%) and female (1.8%) were recorded in 0 – 10 years.

From the results obtained in the antibiogram pattern of the bacterial isolates (Table 3), it was observed that all bacterial isolates were susceptible to nitrofurantoin and resistant to most of the antibiotics. All bacterial isolates were resistant to cefotaxime, ofloxacin, ocefix, cloxacillin and cotrimoxazole. *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed the highest resistance to the commercially used antibiotics while *E. coli* showed the lowest resistance (or highest) susceptibility to the antibiotics as it was susceptible to augmentin, gentamicin, nitrofurantoin, rocephin and askarivid. The antibiogram pattern of these bacterial isolate showed some similarities with the findings of other researchers except in few cases. Oluwu and Oyetunji (2003) showed *S. aureus* to be sensitive to gentamicin and resistant to cotrimoxazole and nalidixic acid. Also, Ahmed and Kudi (2003) reported that *S. aureus* was sensitive to gentamicin and cephaloxin while resistant to cotrimoxazole and penicillin in their work on chronic suppurative otitis media.

Conclusion

It could be said that *S. aureus* is the most frequent etiologic agent of urinary tract infection as shown in this study. There should be a continuous monitoring of bacterial antibiotics susceptibility before antibiotic prescription in order to ensure adequate treatment of urinary tract infection and reduction in the spread of bacterial resistant strain. More also, there is need for a national antibiotics policy as well as a surveillance scheme to ascertain the extent of these consequences of UTIs and antimicrobial sensitivity in our environment.

References


