Antibiotic Sensitivity Profile of Asymptomatic Bacteriuria Isolates from Pregnant Women Attending Antenatal Clinic at Federal Medical Centre Nguru, Yobe State, Nigeria

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Abstract

Background: Urinary tract infection (UTI) during pregnancy is a common problem and is associated with maternal as well as prenatal morbidity and mortality that can be asymptomatic if untreated may lead to symptomatic. Screening and treatment should be practiced, based on urine culture and sensitivity investigation for antibiotics.

Objective: This study was aimed at determining the antibiotic sensitivity profile of asymptomatic bacteriuria isolates from pregnant women attending antenatal clinic at FMC Nguru, Yobe State.

Methodology: A total of one hundred and thirty (130) bacteria isolates were used including: Escherichia coli, 48, (36.9%), Staphylococcus sp.29, (22.3%), Klebsiella sp, 26, (20.0%), Proteus sp, 08, (6.2%), Pseudomonas sp,04, (3.1%) and Streptococcus fecalis, 15, (11.5%). The antimicrobial discs used were based on oral and injectable preventive therapy during pregnancy.

Results: The antimicrobial sensitivity pattern showed that, the isolates were sensitive to Travid (96.9%), Reflacin (90.8%), Ciproflox (93.1%) and Augmentin (99.2%) while most of the isolates are relatively resistant to Ceporex (53.9%), Naldixic acid (61.5%), Gentamicin (39.2%), Streptomycin (46.9), Septrin (21.5%) and Ampicillin (28.5%).

Key words: Asymptomatic bacteriuria, Isolates, Sensitivity, Antibiotics, Pregnant women, Antental

Introduction

Urinary tract infection (UTI) is the most common bacterial infections in the human population, and more frequent infections are observed during pregnancy. Most of the urinary tract infections during pregnancy are asymptomatic, but they could lead to serious health complications such as prematurity, low birth weight, hypertension, and higher fetal mortality rates, if not treated very early with the onset of pregnancy (Moghadas et al.,2009). The combination of mechanical, hormonal and physiologic changes during pregnancy contributes to significant changes in the urinary tract which has a profound impact on the acquisition and natural history of bacteriuria during pregnancy, which usually begins in week 6 and peaks during weeks 22 to 24 of pregnancy due to a number of factors including urethral dilatation, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux (Chaliha et al.; 2002). About 70% of pregnant women usually develop glycosuria, which further encourages bacterial growth in the urine (Al-issa, 2009).

Urinary tract infection (UTI) may manifest as asymptomatic bacteriuria (ASB) or symptomatic bacteriuria (SB). The prevalence of asymptomatic UTI has been previously reported to be 2% to 13% in pregnant women while symptomatic UTI occurs in 1% –18% during pregnancy (Makinde et al., 2009).In a healthy individual the freshly voided urine is sterile and microbe free. Urinary tract infection is more common in females than males due to the shortness of the urethra and is more readily transverse by microorganisms(Uehling, 1999).

Women infected with asymptomatic bacteriuria during pregnancy are more prone to deliver premature or low-birth-weight infants and have a 20- to 30-fold high risk of developing pyelonephritis during pregnancy compared with women without bacteriuria. The presence of a significant quantity of bacteria in a properly
collected urine specimen from a person without symptoms or signs of UTI characterizes as asymptomatic bacteriuria (Schnarr et al., 2008). Untreated UTIs can result into complications, such as pyelonephritis, low-birth weight infants, premature delivery, and occasionally, still birth (Macejko and Schaeffer, 2007). Therefore, prompt treatment of symptomatic UTI and asymptomatic bacteriuria is required in pregnant women. The importance of Coliform bacilli in UTI among pregnant women has long been known in developed countries, and roughly in 80-90% of cases, the most common isolated pathogen is *Escherichia coli* (Le et al., 2004; Christensen 2000; Gilstrap and Ramin, 2001; Ovalle and Levancini, 2001). Other responsible microorganisms include Enterobacteria (*Klebsiella, Enterobacter, Proteus*), *Staphylococcus epidermidis* or *Staphylococcus saprophyticus*, *Enterococcus faecalis* and group B *Streptococcus* (Macejko and Schaeffer, 2007).

Antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographic locates and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of much importance as the changing rate of antibiotic resistance has a large impact on the empirical therapy of Urinary Tract Infections (Taneja et al., 2008).

**MATERIALS AND METHODS**

**Collection of specimen**

This study was conducted in Nguru Local Government and urine samples were collected from pregnant women with the consent attending antenatal clinic at Federal Medical Centre Nguru from May, 2012 to November, 2012. Midstream urine 30 ml - 50 ml was requested from the pregnant women during their first antenatal visit and at least 4 hours stay of urine in bladder was ensured before collection. The samples were processed at Department of Microbiology Federal Medical Centre Nguru, Yobe State.

**Exclusion criteria**

A questionnaire was distributed to the participants to access the bio-data of the participants and exclude women with:-

1. Known congenital anomalies of urinary tract
2. Sign and symptoms of UTI
3. Pyrexia
4. History of antibiotic drugs two weeks before collection of the sample.

**Transportation of the specimen**

Urine sample was collected in a sterile wide-mouth 100ml capacity container with a cover. After collecting and labeling the specimen, it was immediately transported and processed in the laboratory on the same day, in case of any delay, specimen was refrigerated at 4°C. (Cheebrough 2000).

**Culture of midstream urine samples**

The entire urine specimens were properly labeled and the semi quantitative standard wire loop method was employed for the culture. A calibrated wire loop having a diameter of 5mm to deliver 0.002ml of urine was used.

The urine samples were mixed thoroughly before inoculated on Cystein lactose electrolyte deficient (CLED) agar, and Blood Agar plate using the sterile calibrated wire loop, holding the loop upright to avoid more than required volume. All plates were incubated at 35±2°C for 24-48 hours aerobically. (Cheesbrough 2000). A count ≥100,000 colony forming units (CFU) per milliliter of urine was considered positive after incubation and these isolates were identified.

**Test for identification of isolates**

After significant bacteria had been established through the colony count, the isolates were then differentiated through standard Gram’s staining method to gram-negative and gram-positive bacteria. Further identification was carried out through Biochemical characterization on each of the isolates.
Standardization of Inoculum
Using a sterile wire loop, a loopful of colony from a 24 hour culture of the isolates was emulsified in sterile normal saline to match the 0.5 Mcfarland’s standard for sensitivity tests as described by NCCLS (1999).

Antibiotic susceptibility testing
Antibiotic susceptibility test was carried out using the Kirby-Bauer disc diffusion technique on Muller Hinton agar and commercial antibiotic discs (optudisc urine level) were used for antimicrobial testing. The antibiotic discs used were:

- Travid (10 μg)
- Reflacine (10 μg)
- Ciproflox (10mg)
- Augmentin (30 μg)
- Gentamycin (10g)
- Streptomycin (30 μg)
- Ceporex (10mg)
- Naldixic Acid (30mg)
- Septrin (30mg)
- Amplicin (30mg)

The antibiotic disc impregnated culture plates were incubated at 37°C overnight. The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria (NCCLS, 1999).

Results
Out of the three hundred (300) samples, 130 show significant growth while 170 show no any significant growth Table 1 shows a percentage (%) prevalence of 43.3% bacterial growth. Table 2 show the (%) characterization of isolate from the positive culture, where gram negative bacilli are 84 (66.2%) and gram positive cocci are 44 (33.8%). Table 3 show the frequency of the isolate from the positive culture where E. coli is 48 (36.9%); Staphylococcus spp. 29 (22.3%); Klebsiella spp 26 (20.0%); Proteus spp. 8 (6.2%) Pseudomonas spp. 4 (3.1%) and Streptococcal fecalis 15 (11.5%). The sensitivity profile of the isolated urinary tract pathogens from the culture was indicated in Table 4, which shows that all the isolated organisms were sensitive to Travid (96.9%), Reflacine (90.8%), Ciproflox (93.1%) and Augmentin (99.2%) while most of the isolates are relatively resistant to Ceporex (53.9%), Naldixic acid (61.5%), Gentamicin (39.2%), Streptomycin (46.9), Septrin (21.5%) and Ampicilin (28.5%).

TABLE:1  Significant growth of the culture

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant growth</td>
<td>130</td>
<td>43.3</td>
<td>43.3</td>
<td>43.3</td>
</tr>
<tr>
<td>No significant growth</td>
<td>170</td>
<td>56.7</td>
<td>56.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

TABLE:2  Characterization of isolate from the positive sample

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative bacilli</td>
<td>86</td>
<td>66.2</td>
<td>66.2</td>
<td>66.2</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>44</td>
<td>33.8</td>
<td>33.8</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>
### Table 3: Frequency of the Bacteria Isolated in the Significant Culture

<table>
<thead>
<tr>
<th>ISOLATATES</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid percent</th>
<th>Cumulative percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>48</td>
<td>16.0</td>
<td>36.9</td>
<td>36.9</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>29</td>
<td>9.7</td>
<td>22.3</td>
<td>59.2</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>26</td>
<td>8.7</td>
<td>20.0</td>
<td>79.2</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>8</td>
<td>2.7</td>
<td>6.2</td>
<td>85.4</td>
</tr>
<tr>
<td>Pseudomonas spp</td>
<td>4</td>
<td>1.3</td>
<td>3.1</td>
<td>88.5</td>
</tr>
<tr>
<td>Streptococcus fecalis</td>
<td>15</td>
<td>5.0</td>
<td>11.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>43.3</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Missing system</td>
<td>170</td>
<td>56.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Sensitivity pattern of uropathogenic organism to different antibiotics

<table>
<thead>
<tr>
<th>ISOLATED ORGANISMS FROM URINE</th>
<th>E. coli</th>
<th>Staphylococci spp</th>
<th>Klebsiella Spp</th>
<th>Proteus spp</th>
<th>Pseudomonas spp</th>
<th>Streptococcus Spp</th>
<th>ALL SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td>n/N</td>
<td>%</td>
<td>n/N</td>
<td>%</td>
<td>n/N</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Travid (10µg)</td>
<td>48/48</td>
<td>100</td>
<td>29/29</td>
<td>100</td>
<td>25/26</td>
<td>96.2</td>
<td>6/8</td>
</tr>
<tr>
<td>Reflacine (10µg)</td>
<td>38/48</td>
<td>79</td>
<td>29/29</td>
<td>100</td>
<td>26/26</td>
<td>100</td>
<td>8/8</td>
</tr>
<tr>
<td>Ciproflox (10µg)</td>
<td>48/48</td>
<td>100</td>
<td>29/29</td>
<td>100</td>
<td>24/26</td>
<td>92.3</td>
<td>5/8</td>
</tr>
<tr>
<td>Augmentin (30µg)</td>
<td>48/48</td>
<td>100</td>
<td>29/29</td>
<td>100</td>
<td>26/26</td>
<td>100</td>
<td>8/8</td>
</tr>
<tr>
<td>Gentamycin (10µg)</td>
<td>21/48</td>
<td>43.8</td>
<td>13/29</td>
<td>44.8</td>
<td>5/26</td>
<td>19.2</td>
<td>0/8</td>
</tr>
<tr>
<td>Streptomycin (30µg)</td>
<td>3/48</td>
<td>6.3</td>
<td>21/29</td>
<td>72.4</td>
<td>24/26</td>
<td>92.3</td>
<td>0/8</td>
</tr>
<tr>
<td>Ceporex (10µg)</td>
<td>28/48</td>
<td>58.3</td>
<td>17/29</td>
<td>58.6</td>
<td>5/26</td>
<td>19.2</td>
<td>5/8</td>
</tr>
<tr>
<td>Naldixic Acid (30µg)</td>
<td>35/48</td>
<td>72.9</td>
<td>10/29</td>
<td>34.5</td>
<td>21/26</td>
<td>80.8</td>
<td>6/8</td>
</tr>
<tr>
<td>Seprin (30µg)</td>
<td>5/48</td>
<td>10.4</td>
<td>8/29</td>
<td>27.6</td>
<td>3/26</td>
<td>11.5</td>
<td>0/8</td>
</tr>
<tr>
<td>Ampicillin (30µg)</td>
<td>3/48</td>
<td>6.3</td>
<td>6/29</td>
<td>20.7</td>
<td>11/26</td>
<td>42.3</td>
<td>4/8</td>
</tr>
</tbody>
</table>

Key: CR: complete resistance; µg: microgram
DISCUSSION

Asymptomatic bacteriuria are the risk factor predisposing to urinary tract infections more likely during pregnancy which may result into maternal complications such as hypertension, preeclampsia, septicemia resulting in prematurity, low birth weight and higher fetal mortality. Several studies have reported a high incidence of pyelonephritis in pregnant women found positive for bacteriuria (Aseel et al., 2009; Secon et al., 2003)

In the present study, the subjects were from pregnant women attending first antenatal clinic at Federal Medical Centre (FMC) Nguru. Midstream voided urine was taken. The samples were processed for aerobic culture and sensitivity test. The causative organisms of ASB in this study were the same causing symptomatic bacteriuria during fertile life of women as reported by Nathaniel (1985). Asymptomatic bacteriuria is a strong predictor of symptomatic urinary tract infection during pregnancy (MacClean 2001). Significant bacteriuria (10^5 organisms/ml) during pregnancy is an established risk. Factor for other serious complications such as pre-term delivery, fetal Pre-maturity and low birth babies, reported in various studies as 20-40 % (Abbott; 1994) and 66 %. (Mittendorf; 1992, Hodgman; 1994)

The 43.3% bacterial growth obtained in this present study is higher as compared with previous studies, this observation may be attributed to differences in socioeconomic status, multigravida status, maternal age, number of abortions, lifestyle and level of healthcare development (Anuj et al. 2012, Taiwo et al.; 2007). The isolates were subjected to gram staining technique for screening, 86 (66.2%) were found to be Gram negative bacilli and 44 (33.8%) were Gram positive cocci. This finding agreed with the work of Van Norstrand et al. (2000) and Aziz Marjan (2006).

The most prevalent organism isolated in this study is Escherichia coli (36.9%), followed by Staphylococci sp (22.3%), Klebsiella sp (20.0%), Proteus sp (6.2%); Pseudomonas sp (3.1%) and Streptococcus fecalis is (11.5%). The present study has shown the consistency with the previous findings (Mamata et al. 2011). This shows that the etiologic Pattern of Urinary Tract Infections (UTIs) with respect to bacteria pathogens is apparently similar worldwide’. However, Raza et al. (2011), state that the Gram negative aerobic bacteria colonize the uro-epithelial mucosa with adhesion, pilli, and fimbrae.

The antimicrobial disc used was based on oral and injectable preventive therapy during pregnancy as recommended by Delzell et al. (2000). However, the present antibiotic sensitivity profile of the isolates shows that most of the organism were sensitive to Travid (96.6%), Reflacin (90.8%), Ciproflox (93.1%) and Augmentin (99.2%) while most of the isolates are relatively resistant to Ciporex (53.9%), Naldixic acid (61.5%), Gentamicin 39.2%, streptomycin (46.9%), Septrin (21.5%) and Ampicillin (28.5%).

The most effective in-vitro agent in this study was Travid, Riflacine, Augumentin among the injectable and ciproflox among the orally administered drugs. The findings corroborate with the report of, Shamweel et al (2011). Other useful oral and injectable antibiotics are Ceporex, and Naldixic Acid. However, Gentamycin, Septrin, and Ampicillin were not an effective antibiotic in this study. This may be probably due to indiscriminate and uncontrolled use of these antibiotics in our society. The extensive use of these drugs could be attributed to its relatively cheap, easy to administer and often use for emphatic treatment of suspected infections. This facts indicates that urinary pathogens are becoming resistant day by days to commonly used antibiotics in our community’

CONCLUSION

Antimicrobial sensitivity pattern of urine isolates in this study shows resistant to some drugs commonly recommended for safe use during pregnancy. Therefore use of appropriate antimicrobial agents for efficaciy only recommended after culture and sensitivity report, to improve the health care service during pregnancy.

RECOMMENDATION

In view of the above findings, asymptomatic bacteriuria is prevalent among pregnant women attending antenatal clinic at FMC Nguru. Therefore, the following measures are recommended to improve the health care services during pregnancy.
1. Screening pregnant women for bacteriuria and proper treatment should be considered as an essential part of antenatal care in FMC Nguru.

2. Increasing problem of resistant uro-pathogen can be reduced by employing non antimicrobial strategies in the prevention of urinary tract infections (UTIs) such as sufficient fluid intake, complete emptying of bladder during voiding, use of urinary antiseptic and restrictive use of catheter.

REFERENCES

3. Anuj Mathur, Kasturi Mummigatti, Ramesh Ranganathan, Jayadevan Sreedharan (2012); Asymptomatic bacteriuria in pregnant women: A hospital based study. Annual scientific meeting of gulf medical university oral proceeding. 1(S1): S17-S21
22. Raza S, Pandey S, Bhatt CP (2011), Microbiological Analysis of The Urine Isolates In Kathmandu Medical College Teaching Hospital, Nepal: Vol. 9, No.4 Issue- 36
25. Shamweel Ahmad, Shehla Shakooh, Sajad Ahmad Salati, Abdul Munium (2011); Prevalence of asymptomatic bacteriuria among pregnant women in Kashmir: sri Lanka journa og obstetrics and gynaecology; 33: 158-162

