HIGH GENITOURINARY CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG PREGNANT WOMEN ATTENDING ANTE-NATAL CLINICS AT MATER MISERICORDIA HOSPITAL, AFIKPO, EBONYI STATE, NIGERIA.

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is a worldwide human pathogen of enormous public health concern, causing severe morbidity and mortality both in hospitals and the community. Their presence in pregnancy condition is a cause for concern. This study investigated the prevalence and antibiotics susceptibility pattern of urinary methicillin-resistant Staphylococcus aureus among pregnant women attending ante-natal clinics at Mater Misericordia Hospital Afikpo. Midstream urine samples (n=89) were collected from these women and analyzed in the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, using standard microbiological techniques. Fifty-four (60.67%) pregnant women were found to harbor S. aureus and out of these, forty-five (83.33%)
were positive for methicillin-resistance. The results of the antibiotic susceptibility testing revealed varying degrees of susceptibility to amoxicillin (26%), oxacillin (0%), erythromycin (33%), ceftriaxone (4%), clindamycin (33%), meropenem (100%), ofloxacin (44%), gentamicin (62%), tetracycline (17%), cefotaxine (24%), ciprofloxacin (77%) and perfloxacin (71%). The multidrug-resistant index (MDRI) of the MRSA isolates ranged from 0.16 to 0.75. This work revealed high prevalence of MRSA among pregnant women in this area and suggest a possible picture of the state of other pregnant women at the rural communities of the state and the country at large. To avert possible maternal and foetal complications due to this organism, routine screening through the gestation period and prompt treatment of cases are hereby recommended. Also, the need for public enlightenment of the implication of abuse and misuse of drugs cannot be over-emphasize in the fight against drug resistance.

**KEY WORDS:** MRSA, antenatal, multi-drug resistance, prevalence, *Staphylococcus aureus.*

**INTRODUCTION**

Asymptomatic carriage of genitourinary pathogens is a common condition observed in pregnancy, posing enormous maternal, foetal risks and therapeutic challenges (Sabharwal, 2012; Matuszkiewicz-Rowińska et al., 2013; Anozie et al., 2016; Bose et al., 2016). Asymptomatic bacteuria is just slightly higher among pregnant women than their non-pregnant counterparts; however, given pregnancy conditions, if left untreated there is much higher risk (up to 40%) of progression to pyelonephritis, acute kidney injury, anemia, hypertension, sepsis and septic shock, hemolysis, thrombocytopenia, and acute respiratory distress syndrome and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight (Adis Data Information, 2011; Millar and Cox, 1997; Cunningham and Lucas, 1994; Galajdova, 2010; Conde-Agudelo, 2008; Mittal and Wing, 2005; Bolton et al., 2012; Farkash et al., 2012; Gravett et al., 2012; Stanley et al., 2013). Pregnant women are more susceptible to urinary tract infections due to a combination of factors including: short urethra, increased supply of vaginal glycogen and moisture, dilation of the urinary tract combined with slight hydronephrosis, vesico-ureteral reflux and urine retention in the bladder after micturition resulting from the compression of the urinary bladder by enlarged uterus and increased urinary pH among others (Jolley and Wing, 2010; Matuszkiewicz-Rowińska et al., 2013; Anozie et al., 2016). Maternal infections contribute to nearly 40% of all adverse perinatal outcomes (Bolton et al., 2012).
A lot of pathogens have been implicated in urinary tract infections ranging from Gram positive to Gram negative bacteria, with *Escherichia coli* at the leading position. However, several studies have reported that *Staphylococcus aureus* appears to be competing with *Escherichia coli* as a major pathogen responsible for urinary tract infections (UTIs) (Akortha and Ibadin, 2008; Imaide et al., 2010; Ilusanya et al., 2012). *Staphylococcus aureus* is an endogenous microorganism colonizing the skin, mucous membrane, nasal cavities, anuses and vaginal vaults of healthy women (Plata et al., 2009; Onanuga et al., 2005). It is a human pathogen that causes numerous infections ranging from relatively mild skin and soft tissue infections to life threatening sepsis, pneumoma osteomyelitis, urinary tract infections, as well as toxin-mediated syndrome such as toxic shock syndrome and food poisoning (Bal and Gould, 2005; Shittu et al., 2011). It is the essential individual pathogens known to cause infections acquired in hospital and community (Shittu and Lin, 2006).

Commonly accepted antibiotics used in treating UTIs during pregnancy, regardless of its period, include derivatives of penicillin and cephalosporins, particularly those with low protein-binding ability (such as cephalexin), all of United States food and drug administration (FDA) pregnancy category B (Widmer et al., 2011). However, *S. aureus* associated UTIs pose enormous therapeutic challenges due to the growing menace of antimicrobial resistance and the emergence of the strain, Methicillin-resistant *Staphylococcus aureus* (MRSA). Today, MRSA is a worldwide human pathogen of enormous public health concern, causing severe morbidity and mortality both in hospitals and the community, which resist almost all known antibiotics (Nnachi et al., 2014). More than 60% of *S. aureus* isolates are turning to MRSA (Baranovich et al., 2010) and infections by these are more troublesome in nursing homes, penitentiaries, hospitals, invasive devices, wherever patients with exposed wound and debilitated invulnerable systems stand at more danger of nosocomial infection than the universal community.

This is of a serious public health importance as the organism is an opportunistic pathogen and can perpetrate and cause harm at the slightest conducive opportunity to both the unassuming pregnant woman and/or her foetus. Also, the situation is even more grave given the scenario of therapeutic difficulties due to resistance to drugs of choice for UTIs by these organism. This study therefore determined the prevalence and the antibiotic susceptibility profiles of genitourinary MRSA among pregnant women receiving antenatal cares at the Mater Misericordia Hospital, Afikpo.
MATERIALS AND METHODS

Study Setting/Design
This research was carried out at Mater Misericordia hospital, Afikpo in Afikpo North Local Government Area of Ebonyi State among pregnant women attending ante-natal clinic in Mater Misericordia hospital Afikpo, from January-April, 2016. The topographical body known as Afikpo (Ehugbo) is located in the Southern portion of Ebonyi State and it is the second biggest town in the state. It is bounded on the South by Edda, North by Abomege, West by Okposi and East by Abili in Cross River State. Afikpo has an area approximately 164 square kilometers in mass, with the population of 672,000 (National Population Commission, 2006).

Prior to the study, ethical approval was sought and obtained from the Research and Ethics Committee of the Ebonyi State University, Abakaliki, while permission was also obtained from the management of Mater Misericordia hospital, Afikpo. Participation in this study was voluntary; informed consents were obtained from all study participants and pregnant women who denied consents were excluded from the study. All volunteers were not on any antibiotics at the time of sample collection. Early morning midstream urine samples were aseptically collected from eighty-nine (89) pregnant women within 18 to 42 years of age who gave their consents. Structured questionnaire was used to obtain demographic information of each participant including age, occupation, level of education and stage of pregnancy. The collected samples were properly labelled and transported to the Department of Applied Microbiology Laboratory of Ebonyi State University, Abakaliki for processing using standard microbiological techniques.

Inoculation of Plates/Identification of methicillin-resistant S. aureus (MRSA) Isolates
One millilitre (1 ml) of each sample was first inoculated into 9ml of nutrient broth and incubated at room temperature after which growth on nutrient broth was sub-cultured unto Mannitol salt agar plates by streak plate method described by Ani et al. (2015). All plates were incubated at 37°C for 24 hours. After incubation, purity plates were made after which all isolates were identified using a combination of colonial morphology, gram staining, sugar fermentation tests, coagulase test and catalase test (Cheesbrough, 2006). Each isolate of S. aureus was standardized to 0.5 McFarland turbidity (Esimone et al., 2008), after which they were aseptically inoculated on Mueller-Hinton agar plate using sterile swab stick. Single Oxacillin (1µg) and ceftriaxone (30µg) antibiotics were aseptically placed on the surface of
the inoculated plate and incubated for 24 hours at 37°C. The zones of inhibition were measured after incubation and \textit{S. aureus} isolates with zones of inhibition less than or equal to 10 mm were regarded as MRSA according to Clinical Laboratory Standard Institute (CLSI, 2001).

**Antimicrobial Susceptibility Studies**

Antimicrobial susceptibility profiles of the MRSA isolates were determined by Kirby-Bauer (CLSI, 2001) using overnight cultures after adjusting to 0.5 McFarland turbidity. Commercially available single antibiotics discs were aseptically placed on the surface of the inoculated Mueller-Hinton agar (Oxoid, UK). The antibiotics disc used included oxacillin (1µg), ceftriaxone (30µg), clindamycin (15µg), erythromycin (15µg), amoxicillin (25µg), meropenem (10µg), tetracycline (30µg), ofloxacin (5µg), ciprofloxacin (10µg), cefotaxime (30µg), gentamicin (10µg) and pefloxacin (5µg) (Oxoid, UK). The plates were allowed for some minutes to enable pre-diffusion of the antibiotics, after which they were incubated at 37°C for 24 hours. The inhibition zone diameters (IZDs) produced by the antibiotics were measured in millimeter and recorded as suggested by the CLSI (2001).

**RESULTS**

Table 1. Occurrence of \textit{Staphylococcus aureus} and Methicillin-resistant \textit{Staphylococcus aureus} among the pregnant women based on their demographic characteristics

<table>
<thead>
<tr>
<th>Demographic parameter</th>
<th>\textit{S. aureus} Carriage</th>
<th>MRSA Carriage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Examined</td>
<td>No. Positive (%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-22</td>
<td>04</td>
<td>03(75.00)</td>
</tr>
<tr>
<td>23-27</td>
<td>30</td>
<td>20(66.67)</td>
</tr>
<tr>
<td>28-32</td>
<td>35</td>
<td>20(57.14)</td>
</tr>
<tr>
<td>33-37</td>
<td>17</td>
<td>09(53.00)</td>
</tr>
<tr>
<td>38-42</td>
<td>03</td>
<td>02(66.67)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>89</td>
<td>54(60.67)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traders</td>
<td>53</td>
<td>32(60.37)</td>
</tr>
<tr>
<td>Civil servants</td>
<td>14</td>
<td>12(85.71)</td>
</tr>
<tr>
<td>House wives</td>
<td>22</td>
<td>10(45.45)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>89</td>
<td>54(60.67)</td>
</tr>
<tr>
<td><strong>Level of Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>10</td>
<td>3(30.00)</td>
</tr>
<tr>
<td>Primary</td>
<td>4</td>
<td>2(50.00)</td>
</tr>
<tr>
<td>Secondary</td>
<td>42</td>
<td>24(57.14)</td>
</tr>
</tbody>
</table>
Table 2. The Multi-drug Resistance index of 45 Methicillin resistant *Staphylococcus aureus* (MRSA) isolates.

<table>
<thead>
<tr>
<th>MDRI index</th>
<th>No. of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0. $10 - 0.20$</td>
<td>2</td>
</tr>
<tr>
<td>0. $21 - 0.30$</td>
<td>7</td>
</tr>
<tr>
<td>0. $31 - 0.40$</td>
<td>5</td>
</tr>
<tr>
<td>0. $41 - 0.50$</td>
<td>13</td>
</tr>
<tr>
<td>0. $51 - 0.60$</td>
<td>5</td>
</tr>
<tr>
<td>0. $61 - 0.70$</td>
<td>6</td>
</tr>
<tr>
<td>1. $71 - 0.80$</td>
<td>7</td>
</tr>
</tbody>
</table>

The study on the carriage rates and antibiogram of urinary methicillin-resistant *Staphylococcus aureus* (MRSA) among pregnant women attending antenatal clinic at the Mater Misericordia hospital, Afikpo, Ebonyi State, Nigeria revealed that out of the 89 pregnant women sampled, 54 (60.67%) showed the presence of *S. aureus* in their urine. Of this number, 45 (83.33%) harbored MRSA in their genitourinary tract. All the *S. aureus*
isolates from pregnant women within 18-22 and 38-42 years of age were methicillin-resistant. Also, pregnant traders were highest in the carriages of *S. aureus* and MRSA, while 80% of the *S. aureus* isolates from House wives were resistant to methicillin. All the *S. aureus* isolates from pregnant women who had either primary education only or no formal education were methicillin-resistant, while married women had more MRSA than unmarried pregnant women in the study. MRSA carriage was more at the second and third trimesters of gestation (89.47% and 86.67% respectively) (Table 1). MRSA is more susceptible to meropenem, followed by ciprofloxacin and ofloxacin, and resistant to oxacillin, followed by ceftriazone and gentamicin (Figure 1). The multidrug-resistant index (MDRI) of the MRSA isolates lies between 0.75 as the highest and 0.16 as the lowest (Table 2).

**DISCUSSION**

The investigation of the prevalence and antibiotic susceptibility profile of Methicillin-resistant *Staphylococcus aureus* among pregnant women attending antenatal clinics at the Mater Misericordia Hospital, Afikpo, Ebonyi State, Nigeria revealed that 54 of the 89 pregnant women sampled harbored *Staphylococcus aureus* in their urogenital tracts. Forty-five (83.33%) of these isolates of *S. aureus* were Methicillin-resistant. This study supports the report of Akerele *et al.* (2013), who reported that out of 50 *S. aureus* from pregnant women of ages 20-25yrs, 40 (80.00%) were MRSA. The high occurrence of *S. aureus* in pregnant women is as a result of some physiological and hormonal variations observed during gestation which favour the growth of the organism. For instance, at 6th week of pregnancy, the ureter activates to dilation and it remains until delivery. The current study showed that the carriage rate of *Staphylococcus aureus* was highest among pregnant women who were civil servants, and least among pregnant women who were house wives (85% and 45% respectively). However, the carriage rate of MRSA was highest among pregnant women who were traders, and least among pregnant women who were civil servants (88% and 75%) respectively. This could be due to the fact that drug abuse is expected to be higher among traders unlike those in civil service who could be more enlightened due to better level of exposure and education. Most of the pregnant women who were traders agreed to self- and over-the-counter prescription of drugs when ill and discontinued treatment once they felt better. Ehinmidu (2003) observed 50% *S. aureus* among civil servants and 20% MRSA among house wives. The variation in this result could be suggested to be due to differences in the study area and the level of enlightenment of the study population. This is further explained by the fact that, although prevalence of *Staphylococcus aureus* in this work was
highest among the pregnant women who had tertiary level of education (75%) and least among pregnant women without formal education (30%), the prevalence of MRSA was highest among pregnant women with only primary level of education and those without formal education, but least among pregnant women with tertiary level of education. Chen et al. (2006) reported 40% carriage of *S. aureus* among pregnant women with tertiary level of education and 50% MRSA among pregnant women without formal education. With respect to stage of pregnancy, this study observed that occurrence of *S. aureus* was highest amongst expectant mothers who were in their second trimester (67%) and least in third trimester of gestation. On the other hands, the prevalence of MRSA was highest among the pregnant mother who are in their second trimester of gestation (89%) and least among pregnant women who were in their first trimester of gestation (75%). This is in agreement with Gordon (2008) who observed 85% MRSA among pregnant women who were in their second trimester and Chen et al. (2006) who found 74% among pregnant women who are in their first trimester. In this study, the carriage level of *S. aureus* was found to be 61% and 33% for married and unmarried pregnant women respectively, which shows a difference in significant (P<0.05) in the married and unmarried women. This is in agreement with Ehinmidu (2003) who observed 60% and 33% *S. aureus* in urine samples of both married and unmarried pregnant women respectively, signifying that marital status is a remarkable factor in the colonization of *S. aureus* infection.

Antibiotic profile of the MRSA isolates showed complete resistance to oxacillin, ceftriaxone, gentamicin and tetracycline, supporting that all β-lactam antibiotics are being resisted by strains of MRSA (Weems, 2001; Nnachi et al., 2014). The high resistance (100%) shown to oxacillin is bothersome as it commonly forms the first line treatment for most persons with indicators of staphylococcal infections and has been supposed to be a wide-ranging spectrum antibiotic for gram-positive organisms. It is also assumed safe in gestation. Amoxicillin, gentamicin and tetracycline are the most common “over-the-counter” antibiotics which can clarify that the high resistance reached by isolates is associated with drug mishandling/indiscriminate use of drug and self medication, along with poor hygiene and infection control which are extremely prevalent in Nigeria (Akerele, et al., 2013).

**CONCLUSION**

This work revealed high prevalence of MRSA among pregnant women in this area and suggests a possible picture of the state of other pregnant women living in the rural
communities of the state and the country at large. This study observed that a great proportion of these women were asymptomatic carriers, making them potential reservoirs for spread of infection. To avert possible maternal and foetal complications due to this organism, routine screening through the gestation period and prompt treatment of cases are hereby recommended. Also, the need for public enlightenment on the implication of abuse and misuse of drugs cannot be over-emphasize in the fight against drug resistance. Further studies are required to test for significant bacteuria so as to proper associate the MRSA carriage with urinary tract infections during pregnancy.

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