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Prevalence and Antibiotic Susceptibility of *Staphylococcus Aureus* and Other *Staphylococcal* Infections in Pregnant Women Attending Antenatal Clinic in a Tertiary Hospital in Port Harcourt, Nigeria

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Abstract

Background: Infections due to *Staphylococcus aureus* have been on the increase globally with serious implications for public health. Both adults and children can be affected. Although *S. aureus* commonly resides in the nose of apparently healthy humans, it can also colonize such other areas as the intestine, vagina, groin and armpit. It is known to cause asymptomatic and sometimes uncomplicated skin infections but has also been implicated in serious diseases such as endocarditis and toxic shock syndrome. Mounting evidence appears to support increasing *Stapylococcus aureus* colonization and infection among pregnant and postpartum women as well as neonates. Other members of the genus *Staphylococcus* have also been increasingly implicated as causative agents for a variety of disease conditions. In developing countries and resource poor settings, due to lack of adequate facilities or cost, staphylococcal isolates may not be definitively identified to the species and strain level. The result is that other members of the genus *Staphylococcus* may be erroneously identified as *Staphylococcus aureus*. Besides the obvious negative impact this practice may have on chemotherapeutic outcome and antibiotic resistance resulting from misuse of these drugs, the true prevalence of the various staphylococcal pathogens, especially *Staphylococcus aureus*, may not be known particularly with respect to pregnant women.

Objectives: We set out to determine the prevalence and antibiotic susceptibility of *Staphylococcus aureus* and other staphylococcal organisms in pregnant women and those of child bearing age at the University of Port Harcourt Teaching Hospital (UPTH) in Port Harcourt, Nigeria. We determined to identify every isolate to the species and strain level in order to establish their true prevalence and antibiotic susceptibility as well as any significant association between pregnancy status and rectovaginal colonization.

Method: We carried out a cross sectional prospective study involving 265 pregnant women attending antenatal clinic and 242 non-pregnant women attending the general outpatient clinic in the teaching hospital in Port Harcourt, Nigeria. High vaginal swab samples were aseptically collected from each participant and cultured using standard microbiological and biochemical methods. The isolated organisms were identified using the Biomieriux API staph® testing system and revealed a variety of staphylococcal species besides *Staphylococcus aureus*. Antibiotic susceptibility testing was done using the Bauer-Kirby disk diffusion method.

Results: Staphylococcus aureus was clearly the most common organism isolated with a prevalence of 6.9% and 7.7% in the pregnant and non-pregnant women respectively. The difference in the prevalence of Staphylococcus aureus in the pregnant and non-pregnant women was not statistically significant ($p \leq 0.50$). The antibiotic susceptibility testing results showed multiple drug resistance by the organisms isolated. A high level of resistance to the fluoroquinolones and cefixime a third generation oral cephalosporine was observed. Interestingly, a significant difference was seen in the susceptibility of isolates in the pregnant women to erythromycin when compared to those in the non-pregnant women ($p \leq 0.50$)

Conclusion: There is an increasing colonization and infection of the female reproductive tract by *Staphylococcus aureus* and other staphylococcal pathogens. Since these pathogens have been known to be transferable from infected mother to her infant either during or after birth and considering their virulence potential, there is need to take a closer look at the rate of rectovaginal colonization by these organisms in this population and their possible contribution to maternal and infant health.

Keywords: *Staphylococcus aureus*, prevalence, pregnant women, multiple drug resistance, antibiotic susceptibility testing, Nigeria.

Introduction

There are over 30 species currently recognized in the genus *Staphylococcus* [1].These organisms are ubiquitous and very often exist as commensals on the skin and mucous membranes causing no apparent harm to the host. However, they can also colonise other parts of the body sometimes causing life threatening diseases especially when the body's immune status has been compromised [2].

The pathogenicity of *Staphylococcus aureus* has long been established [3]. It is by far the most important and virulent pathogen among the staphylococci and can cause disease in otherwise healthy individuals [4]. Apart from skin and soft tissue infections (SSTIs), *Staphylococcus aereus* can cause such other serious infections as bacteremia or sepsis, staphylococcal pneumonia, bacterial endocarditis, osteomyelitis and

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toxic shock syndrome [5]. Staphylococcal sepsis is a leading cause of shock and circulatory collapse and if untreated *Staphylococcus aureus* sepsis carries a high mortality rate (11-43%) especially in cases of severe and extensive burns [3-6]. *S. aureus* has also been reported as a causative factor, although on a small scale, for chorioamnionitis and neonatal sepsis in pregnancy [7,8]. *S. aureus* is an important cause of both healthcare- and community - associated infections [9-11] having acquired resistance to most antibiotics currently in use thus complicating the treatment of *S. aureus* infections [12].

Staphylococcus aureus has the ability to coagulate blood using the coagulase enzyme and on the basis of this can be distinguished from most other coagulase negative staphylococcal species such as *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis and Staphylococcus lugdunensis* [1,13,14]. Among the coagulase -negative species, distinct types of infections and patterns of antimicrobial susceptibility have been noted [15,16]. While *Staphylococcus saprophyticus* is commonly associated with community acquired urinary tract infections [14, 16], *Staphylococcus lugdunensis* being particularly virulent, has been implicated as a notable cause of destructive endocarditis [15]. For this reason, identification of clinical isolates to the species and strain level is increasingly of clinical and epidemiological importance [3].Unfortunately this is not often done due to cost and lack of facilities resulting in wrong reporting of other species as *Staphylococcus aureus* and further compounding the problem of antibiotic resistance especially in developing countries and resource poor settings.

A number of the studies done on *Staphylococcus aureus* both in Nigeria and elsewhere have focussed on nasal carriage of the organism and its impact on skin and soft tissue infections [17]. Nasal carriage, however, has been reported to predispose for blood borne, surgical and nosocomial infections [18, 19]. Some other studies done in our region have also focussed on the role of *Staphylococcus aureus* in urinary tract infections (UTIs) and their findings show that *Staphylococcus aureus* appears to be competing with *Escherichia coli* as a major pathogen responsible for UTI*s* [20-22]. In fact in two different studies *Staphylococcus aureus* was found to be the predominant pathogen isolated ahead of *E.coli* and Klebsiella species in urinary tract infections [20,22]. Asymptomatic bacteriuria if left untreated can predispose to acute cystitis and pyelonephritis in pregnancy and the increasing colonization of the urinary tract by *Staphylococcus aureus* implies potential threat in pregnancy [23] .There is mounting evidence in support of increasing *Staphylococcus aureus* colonisation and infection in pregnant and post partum women as well as in healthy neonates and hospitalised infants in intensive care units [24]. It has been further established that infants born to mothers with staphylococcal colonization had a greater likelihood of being colonized probably through early postnatal acquisition [25]. Some known risk factors for *Staphylococcus aureus* colonization in the infant include breastfeeding and the size of the household [26]. Although it is not yet clear what role maternal nasal and anogenital colonization plays in infant colonization, it appears certain that *S. aureus* infections are more frequent among those colonized with this pathogen in the anterior nares and elsewhere [18, 19, 27]. To the best of our knowledge, no study has been undertaken in our region to establish the prevalence of *Staphylococcus aureus* and other staphylococcal infections in pregnancy. Antibiotic susceptibility of staphylococcal isolates from other sites have been done [21,22]but results could differ for those isolated from the reproductive tract of pregnant women. There is therefore a paucity of epidemiological data necessary for planning and implementation of relevant health policies.

It is in the light of this that this cross sectional study looks at the prevalence and antibiotic susceptibility of *Staphylococcus aureus* and other staphylococcal infections in pregnancy.

Methods

We performed a cross-sectional prospective study of pregnant women and non-pregnant women of child bearing age infected with *Staphylococcus aureus* and other staphylococcal pathogens colonizing the reproductive tract. The study was conducted at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria from May 11 to September 12, 2011. Approval for the study was granted by the Research and Ethics Committee of the hospital following the submission of a detailed proposal of the intended study and subsequent application for ethical approval.

Subjects

Briefly, 265 pregnant women in different trimesters and 242 non pregnant women between the ages of 18 and 45 years were recruited into the study from May 11 to September 12, 2011. A member of the research team, with the permission and assistance of the Matron in charge of the unit and the chief resident, addressed attendees at the clinic and informed them of the proposed study. The objectives, methods, benefits and possible risks as well as rights of participants and the need to give their informed voluntary consent were generally explained to them. They were then allowed to ask questions where they had doubts and these were adequately addressed to their satisfaction. This general discussion was then followed by a detailed individual counselling conducted privately by the gynaecologist in the office where those who gave informed voluntary consent were assisted to fill out a structured questionnaire. The signed consent form and the filled questionnaire were carefully assigned a unique reference number and dated.

Inclusion Criteria: Those enrolled into the study were asymptomatic apparently healthy pregnant women and non-pregnant women with no known medical complications as assessed by the consultant gynaecologist and who were within the required age range.

Exclusion Criteria: Women with HIV/AIDS, certain medical conditions such as diabetes and other complications that may predispose them to infections were excluded. Those who declined to give their informed voluntary consent were also excluded.

Data collection

The socio-demographic data of all consenting participants were collected by means of a standard structured questionnaire. To maintain confidentiality and improve the accuracy of responses, names and addresses of respondents were not mandatorily required. Socio-demographic data collected included maternal age, gestational age, previous obstetric history, history of current pregnancy, religion, marital status, type of marriage (monogamy or polygamy), occupation, educational qualification, gravidae (prima or multi-gravidae), parity and history of antibiotic usage prior to enrolment in to the study [28].

Specimen collection

The high vaginal swab specimens were collected by House Officers who had been specifically trained by the consultant gynaecologist for this purpose. Briefly the exterior part of the vagina was cleaned with Savlon solution. Then a sterile disposable cosco vaginal speculum was carefully inserted to expose the cervical os and a sterile swab stick was used to collect a high vaginal specimen which was properly labelled with the same unique reference number on the participant's signed consent form and questionnaire. All collected samples were then sent to the microbiology laboratory for analysis. Prior to sample collection

Gram Positive Disc	Code	Concentration (µg)	Gram negative Disc	Code	Concentration (µg)
Erythromycin	E	10	Nitrofurantoin	N	100
Cetriaxone	CT	30	Cetriaxone	CT	30
Ampicillin	AP	30	Ciprofloxacin	CIP	10
Cefixime	CE	5	Gentamicin	GN	10
Levofloxacin	LV	5	Ofloxacin	OF	10
Norfloxacin	NB	10	Augumentin	AU	30
Ciprofloxacin	CIP	5	Pefloxacin	PF	30
Gentamicin	GN	10	Clarithomycin	CM	30
Ofloxacin	OF	5	Chloramphenicol	C	10
Clindamycin	CD	10	Ampicillin	AM	30

Table 1: Antibiotics in the disc used for antibiotic susceptibility tests.

for this work, a pilot study had previously been done to ensure standardization of tests and quality control with respect to organisms, media and antibiotic discs used for susceptibility testing.

Laboratory methods

Methods used included standard culture, microscopy, biochemical assays and antibiotic susceptibility testing using appropriate culture media and reagents.

Using streak plate method, the high vaginal swab specimens were streaked on to blood agar, chocolate agar and MacConkey agar and incubated at 37°C for 24 hours. For samples that showed growth isolates were taken and Gram stained to determine whether they were Gram positive or Gram negative organisms. The isolates were further tested for catalase, coagulase and oxidase activity using standard methods. Gram positive, oxidase-negative isolates were further plated on Baird Parker and Mannitol salt agar to check for Staphyloccocal species. Isolates were emulsified in API Staph medium (Biomerieux®, UK) for staphylococcal species or API Rapid 32 (Biomerieux®, UK) for Gram negative, oxidase negative enterococcal species and the results read manually. API WEB™ stand alone software (Biomerieux, UK) Version 1.2.1 was used to confirm the identity of the organisms isolated and compared with manually obtained results.

Antibiotic susceptibility testing

The susceptibility of the staphylococcal isolates to some antibiotics including erythromycin, ceftriaxone, ampicillin, cefixime, levofloxcin, norfloxacin, ciprofloxacin, gentamicin, ofloxacin clindamycin, nitrofurantoin, augmentin, perfloxacin, clarithromycin, and chloramphenicol was determined using the disk-diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute [29]. The concentrations of the various antibiotics are shown in Table 1.

Data and statistical analysis

All data generated were analysed using statistical package for social sciences (SPSS) version 17.0 and Microsoft Excel 2007. Results were considered statistically significant at 5% level of significance ($p \leq 0.05$).

Limitations of the study

Since this was a hospital based cross sectional study, extending the duration of the study and getting a larger sample size would have enabled us arrive at inferences and conclusions that would be more statistically significant and widely applicable but due to lack of funding this was not possible.

Results

All the results are shown in Tables.

Discussion

The socio-demographic data of the study participants is shown in Table 2a-2l and reveal a very sexually active population. Although the mean age at marriage was 25.22years +/- 8.5, it could be seen that the women actually became sexually active long before they were married as shown by the age at sexual debut of 20.74years +/- 6.2. Young age at first sexual intercourse, early marriage and very active sex life are known to predispose to reproductive tract infections and other pathogens such as *Staphylococcus aureus* that may be pushed up the reproductive tract

Table 2: Socio-demographic characteristics of study participants.

Table 2a: Mean Age of the Women both pregnant and non-pregnant.

Mean Age of the women	30.62yrs +/- 8.5
Mean Age at Marriage	25.22yrs +/- 5.6
Mean Age at First Sexual Intercourse	20.74yrs +/- 6.2

Table 2b: Age Ranges of Pregnant and Non- Pregnant women.

	Pregnant	Non-pregnant
Age (years)		
10-19	-	1(0.4)
20-29	152(57.4)	95(39.3)
30-39	112 (42.3)	94(38.8)
40-49	1(0.4)	52(21.5)

Table 2c: Marital Status of Pregnant and Non-Pregnant women.

	Pregnant	Non-pregnant
Marital status		
Single	14 (5.3)	94 (38.8)
Married	242 (91.3)	135 (55.8)
Divorced	7(2.6)	5 (2.1)
Separated	2 (0.8)	-
Widowed	-	8 (3.3)

Table 2d: Age at Marriage of Pregnant and Non-Pregnant Women.

	Pregnant	Non-pregnant
Age married (years)		
10-15	2 (0.8)	25 (10.3)
16-20	12 (4.5)	13 (5.4)
21-25	109 (41.1)	50 (20.7)
26-30	110 (41.5)	45 (18.6)
>30	29 (10.9)	19 (7.8)
Not applicable	3 (1.1)	90 (37.2)

Table 2e: Age at First Intercourse of Pregnant and Non-Pregnant Women.

	Pregnant	Non-pregnant
Age at first intercourse (years)		
10-15	5 (1.9)	10 (4.1)
16-20	106 (40.0)	90 (37.2)
21-25	105 (39.6)	95 (39.3)
26-30	45 (17.0)	30 (12.4)
>30	4 (1.4)	3 (1.2)
Not applicable	-	14 (5.8)

Table 2f: Type of Marriage of Pregnant and Non-Pregnant Women.

	Pregnant	Non-pregnant
Marriage type		
Single	3 (1.1)	86 (35.5)
Monogamous	262 (98.9)	129 (53.3)
Polygamous	-	12 (5.0)
Others	-	15 (6.2)

Table 2g: Religion of Pregnant and Non – Pregnant Women.

	Pregnant	Non-pregnant
Religion		
Christian	259 (97.7)	230 (95.4)
Islam	1 (0.4)	2 (0.8)
Others	5 (1.9)	9 (3.7)

Table 2h: Educational status of Pregnant and Non-Pregnant Women.

	Pregnant	Non-pregnant
Education		
No formal education	4 (1.5)	3 (1.2)
Primary	70 (26.4)	10 (4.2)
Secondary	149 (56.2)	84 (35.0)
Tertiary	42 (15.8)	143 (59.6)

Table 2i: Occupation of Pregnant and Non-Pregnant Women.

	Pregnant	Non-pregnant
Occupation		
Student	43 (16.3)	84 (31.7)
Civil servant	75 (28.4)	65 (26.9)
Trader	72 (27.3)	55 (22.7)
Housewife	74 (28.0)	38 (15.7)

Table 2j: Parity of Pregnant and Non-pregnant Women.

	Pregnant	Non-pregnant
Parity		
0	111 (41.9)	122 (50.4)
1	62 (23.4)	21 (8.7)
2	50 (18.9)	23 (9.5)
3	11 (4.2)	18 (7.4)
4	8 (3.0)	18 (7.4)
≥5	3 (1.1)	18 (7.4)
Not applicable	20 (7.5)	22 (9.1)

Table 2k: Gestational Age of Pregnancy.

	Pregnant	Non-pregnant
Gestational age		
1st trimester	56 (21.2)	-
2nd trimester	122 (46.0)	-
3rd trimester	87 (32.8)	-
Not applicable	-	242 (100.0)

Table 2l: Frequency of Sexual contact/week of Pregnant and Non-Pregnant Women.

Frequency of sexual contact/week	Pregnant	Non-pregnant
NONE	6 (2.3)	6 (2.5)
Once	48 (18.1)	71 (29.3)
Twice	84 (31.7)	63 (26.0)
Thrice	43 (16.2)	34 (14.0)
Four times	5 (1.9)	11 (4.5)
≥ 5 times	7 (2.6)	10 (4.1)
Not applicable	72 (27.2)	47 (19.4)

during sexual activity [30]. The data also showed study participants with a high literacy level as 72% and 94.5% of the pregnant and non-pregnant women respectively had at least a secondary education. Civil servants and traders constituted 55.7% and 49.6% of the number of pregnant and non-pregnant women respectively meaning that these women enjoyed reasonable financial independence with an improved socioeconomic status. A statistically significant relationship was seen between literacy level and socioeconomic status and infection ($p \leq 0.05$). This could account for the low prevalence of *Staphylococcus aureus* and other infections seen in this study since poor economic status has long been established as a predisposing factor for UTIs and reproductive tract infections [31,32]. Multiparity is also a risk factor that may contribute to the acquisition of asymptomatic bacteriuria in pregnancy [33,34]. However, only 4.1% of the pregnant women in this study had 4 or more children and this may have minimized the risk of Multiparity to bacterial colonization. Sexual activity and certain methods of contraception are also said to increase the risk of bacteriuria in pregnancy [35] and though the women in this study were sexually very active as shown by the number of sexual contacts per week in Table 2xii, this did not seem to translate to increase in infection rate. This may be due to higher level of hygiene resulting from the high level of literacy and socioeconomic status of the study participants.

The type and frequency of occurrence of staphylococcal organisms isolated from both pregnant and non-pregnant women are shown in Table 3. Several members of the genus *Staphylococcus* were isolated from the study population. The prevalence of the organisms isolated is recorded in Table 4. *Staphylococcus aureus* was predominant isolate followed by *Staphylococcus xylosus* and *Staphylococcus haemolyticus*. Many laboratories especially in developing countries such as Nigeria may not always be able to definitively identify clinical isolates due to paucity of facilities and funding. The result is that often other members of the genus are wrongly identified as *Staphylococcus aureus*. Even though *Staphylococcus aureus* is known to be the most virulent member of this genus [36,37], other coagulase –negative staphylococci are also known to be pathogenic to varying degrees and this underscores the need to identify the isolates to the species and strain level [3]. *Staphylococcus haemolyticus,* a coagulase negative *Staphylococcus* can be found as normal skin flora and is the second most common coagulase-negative staphylococci isolated in human blood [38]. It is a major cause of nosocomial infections that has been implicated in septicaemia, peritonitis, infections involving the urinary tract and has also been linked to a case of infective endocarditis [39]. *Staphylococcus haemolyticus* is an opportunistic pathogen made remarkable by its ability to alter its genome content and to acquire resistance to antibiotics [40].

Staphylococcus sciuri causes endocarditis, peritonitis, septic shock and urinary tract infections while *Staphylococcus epidermidis* is the most commonly isolated pathogen in nosocomial infections with critically ill immunocompromised patients and premature neonates being most vulnerable [41].

Staphylococcus aureus and non- *aureus* coagulase- negative staphylococci possess a remarkable ability to acquire resistance to multiple antibiotics and the obvious clinical implication of this is limited

Organism	Pregnant women	Non-Pregnant	Frequency	Percentage of total frequency
Staphylococcus aureus	35	39	74	72.5
Staphylococcus hominis	4	-	4	3.9
Staphylococcus sciuri	-	2	2	2.0
Staphylococcus xylosus	1	7	8	7.8
Staphylococcus haemolyticus	5	-	5	4.9
Staphylococcus epidermidis	1	1	2	2.0
Micrococcus luteus	1	2	3	2.9
Kocuria varians	2	2	4	3.9

Table 3: Types of staphylococcal organisms isolated from the pregnant and non-pregnant women and their frequency of occurrence.

Organisms	Pregnant women	Prevalence	Non-pregnant women	Prevalence
Staphylococcus aureus	35	6.9%	39	7.7%
Staphylococcus hominis	4	0.8%	-	-
Staphylococcus sciuri	-		2	0.4%
Staphylococcus xylosus	1	0.2%	7	1.4%
Staphylococcus haemolyticus	5	0.2%	-	-
Staphylococcus epidermidis	1	0.2%	1	0.2%
Micrococcus luteus	1	0.2%	2	0.2%
Kocuria varians	2	0.4%	2	0.4%

Table 4: Prevalence of Staphylococcal pathogens isolated from pregnant and non-pregnant women.

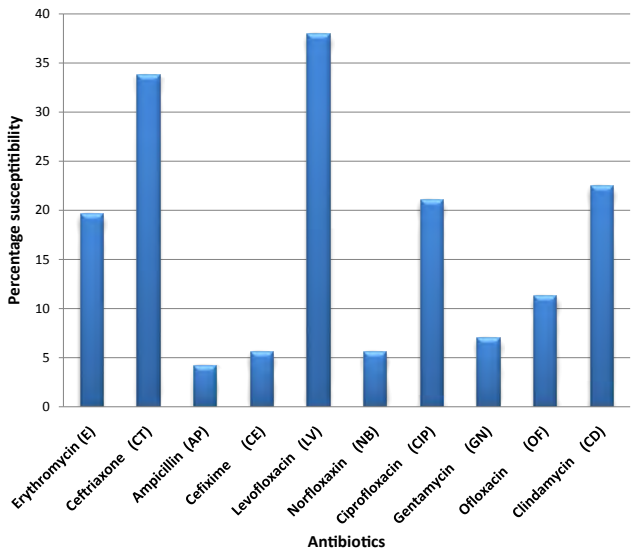

Figure 1: Percentage susceptibility of Staphylococcus aureus to tested antibiotics.

therapeutic options with attendant increase in mortality and morbidity [42]. The options for chemotherapy are further narrowed in pregnancy when some effective drugs may be contraindicated and this makes the high prevalence of *Staphylococcus aureus* observed in this study all the more worrisome and the need to find solutions more imperative.

Staphylococcus aureus was the dominant pathogen isolated in this study and this is in agreement with previous studies [20-22] which had a similar finding. However, the prevalence of 6.9% in pregnant women and 7.7% in non pregnant women seen in our study was much lower than the 24. 4% obtained by Imade et al and 22.8% obtained by Akortha and Ibadin. This may be attributable to the higher socioeconomic and educational status of those assessing antenatal services at the UPTH which is a tertiary hospital as well as the stringent measures applied in conducting the laboratory tests.

The results of the antibiotic susceptibility tests on the isolated organisms revealed that many of the isolates were only moderately susceptible to the antibiotics tested. Since *Staphylococcus aureus* was clearly the most common organism isolated, its antibiotic susceptibility profile is presented in Figure 1. The organism was moderately susceptible to laevofloxacin, ceftriaxone , clindamicin and erythromycin in decreasing order. This agrees in part with Imade et al [21] who found ceftriaxone, ciprofloxacin and augmentin to be effective against *S.sureus* but disagrees with Akortha and Imadin who recommended augmentin, ofloxacin, and gentamicin as drugs of choice for *S. aureus* With the exception of laevofloxacin, *Stapphylococcus aureus* showed considerable resistance to the fluoroquinolones such as ciprofloxacin, ofloxacin and norfloxacin. This may be due to the fact these are older and cheaper drugs *than* laevofloxacin and may be prone to misuse and abuse.Another striking observation besides very poor susceptibility of *Staphylococcus aureus* to many of the floroquinolone antimicrobial agents was the resistance of S. *aureus* to third generation oral cephalosporin cefixime. The fluoroquinolones are commonly used antimicrobial agents in clinical practice, sometimes empirically, and the development of resistance to these agents by members of the staphylococci will further deplete the options available for chemotherapy.

Conclusion and Recommendation

The prevalence of *Staphylococcus aureus* infection was 6.9% and 7.7% in pregnant and non-pregnant women respectively. There was no statistically significant difference in the prevalence in the pregnant as well as in the non-pregnant women ($p \leq 0.05$). The *S. aureus* isolated was susceptible to laevofloxacin, ceftriaxone, clindamicin and erythromycin but resistant to fluoroquinolones such as ciprofloxacin and to cefixime a third generation oral cephalosporin. The isolation of other pathogenic non- *aureus* coagulase-negative staphylococci such as *Staphylococcus xylosus* and *Staphylococcus haemolyticus* necessitates efforts at complete and confirmative identification of isolates to the strain level. This will ensure targeted prescribing by clinicians aimed at minimizing the development and spread of antimicrobial resistance.

Although the *S. aureus* prevalence seen in this study is relatively low, there is need for further investigation into the extent and impact of *S. aureus* infection in pregnancy and maternal health. It is also recommended that *Staphylococcus aureus* isolated from the reproductive tract be tested for methicillin resistance and that further studies be undertaken to establish the direct contribution of *Staphylococcus aureus* and other members of the genus staphylococci to women's reproductive health.

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