Prevalence of *Staphylococcus aureus* among Female Patients attending Rivers State University Teaching Hospital (RSUTH) in Port Harcourt, Nigeria

ABSTRACT

Objectives: To isolate, identify and determine the prevalence of *Staphylococcus aureus* among Female Patients attending Rivers State University Teaching Hospital in Port Harcourt using High Vaginal Swab samples.

Methods: A cross-sectional study was carried out on total of 103 female patients attending the outpatients department and Antenatal clinic of Rivers State University Teaching Hospital. High Vaginal Swab samples were aseptically collected from 55 non-pregnant and 48 six weeks postpartum women. These were then analysed in the Microbiology Laboratory of the Hospital, following standard microbiological procedures. Questions on age, number of sexual partners, knowledge of vaginal infection, symptomatic case, History of previous infection and treatments was responded to by each participant through the completing of questionnaires. Mannitol Salt Agar (MSA) was used for the isolation of the organism and their phenotypic traits were determined based on their cultural and biochemical characteristics.

Results: The study revealed that 25 (24.3%) consisting of 20 (36.4%) non-pregnant women and 5 (10.4%) Postpartum women were positive for *Staphylococcus aureus* infection. The prevalence of *S. aureus* among different groups was statistically significant, (*P*= .002). The highest percentage of *S. aureus* isolation (36.4%) was found in non-pregnant population. Prevalence of *S. aureus* was found highest 10 (18.2%) in 21-25 age group among non-pregnant women and none in 26-30 age group among postpartum women. Data obtained from the questionnaires returned showed that 10 (40%) that tested positive for the organism were asymptomatic as at the time of sample collection, had history of previous reproductive tract infection and had previously undergone treatment.

Conclusion: The high rate of vaginal *Staphylococcal* colonisation observed and the positive correlation between *Staphylococus aureus* prevalence and age, Child delivery status as well as treatment of previous reproductive tract infection seen in the study suggests the need for awareness on the importance of personal hygiene, proper antibiotic usage and regular screening of females within reproductive age.

KEYWORDS: Prevalence, *Staphylococcus aureus,* Female Patients, Port Harcourt.

1.0 INTRODUCTION

Staphylococcus aureus is a major contributor to most reproductive and obstetric disorders (Sulagna *et al*., 2020). It is the most pathogenic *Staphylococci* species that is responsible for divers' disease conditions such as food poisoning, boils, septicemia, pustules, soft tissue infections, urinary tract infections (UTIs), impetigo, osteomyelitis, mastitis, meningitis, bronchopneumonia and wound infections, in both immunocompromised hospitalized persons and healthy immuno-competent people (Tong *et al.,* 2015). *Staphylococcus aureus* has been shown to frequently colonize the vagina of females of reproductive age, specifically 11-60 years (Mumtaz *et al*., 2008). A major cause of aerobic vaginitis, a type of bacterial vaginosis, is *S. aureus,* along with *Ureaplasma urealyticum*, *Mycoplasma hominis*, *E. coli,* and *Pseudomonas* species (Wondemagegn, *et al*., 2015; Abdelaziz *et al.,* 2014). Worldwide, between 5 and 10 million women seek gynecologic assistance for vaginitis each year (Donder *et al*., 2002). Since, bacterial vaginosis is seen as a moderate, non-life-threatening condition, it has been handled lightly over the years. However, it is a morbid condition that makes it easier to contract and spread a variety of STIs, including gonorrheae, trichomoniasis, herpes simplex virus type 2 (HSV-2) and Human Immunodeficiency Virus (HIV) (Myer *et al*., 2005). Other complications has also resulted from bacterial vaginosis, these include; miscarriage, preterm labour, preterm delivery, and postpartum endometritis (Hay *et al.,* 1994; CDC, 2006). As an opportunist organism, *S. aureus* can enter the tissue by breaking through the skin and mucous membrane barriers via traumatic inoculation causing a local abscess lesion, and then spread to the bloodstream resulting in septicaemia (Thomer *et al*., 2016). *Staphylococcus aureus* is also capable of producing extracellular toxins such as exfoliative toxins A and B, toxic shock syndrome toxin-1 (TSST-1), and enterotoxins A to E (Mehrotra *et al.,* 2000). By producing toxins, it can cause life-threatening complications like toxic shock syndrome in menstruating women especially in those who use tampons. Studies conducted in 1980–1981 found that 25–40% of *S. aureus* strains produce Toxic Shock Syndrome Toxin 1 (TSST–1) and that 10-15% of women has vagina *S. aureus* colonization (Schlievert *et al.,* 2007). This study therefore, is intended to evaluate the spread of *Staphylococcus aureus* among women of reproductive age in Port Harcourt, Nigeria.

2.0 METHODS

2.1 Description of Study Area

The study was conducted at the Rivers State University Teaching Hospital (RSUTH) in Port Harcourt Local Government Area of Rivers State, Nigeria. RSUTH formerly known as Braithwaite Memorial Specialist Hospital (BMSH) is situated at Port Harcourt, the capital of Rivers State which is the largest Local Government Area of the State in terms of population (National Bureau of Statistics, 2010). It is one of the major Hospitals in Port Harcourt city that is frequently patronised by the Rivers people.

2.2 Study Population

The group of interest in the study were female patients above fourteen years of age attending Outpatients department and Antenatal Clinic of the Hospital.

2.3 Inclusion Criteria: Only Female patients within the required age group, not on any antibiotics and accept by signing the informed consent form to participate in the study were included.

2.4 Exclusion Criteria: Women below Fourteen years of age that are on any antibiotics and decline to participate in the study were not included.

2.5 Sample Size Determination

Using the Cochran formula ($n=Z^2*p(1-p)/d^2$), the sample size of 103 was determined (Chanuan *et al.*, 2021).

Where,

n= Sample size

Z=Z-score (1.96)

P=Estimated proportion of the population with the attribute in question based on previous study (7.2%) (Gizachew *et al.*, 2015)

d=Margin of error (5%)

2.6 Specimen Collection

Collection procedure was explained and self-administrable vaginal swab sticks along with questionnaires having an assigned unique reference code were given to each participant for the collection of high vaginal swab sample and vital information. Samples were properly labelled with same code on each participant questionnaire and then transported to the Microbiology laboratory of the Hospital.

2.7 Isolation of Organism

High vaginal Swab samples were inoculated onto Mannitol salt agar medium using streak plate method and incubated in inverted position at 37^{o}C for 24 hours (Cheesbough, 2000).

2.8 Characterisation of *Staphylococcus aureus* Isolates

Isolates were characterised based on their cultural morphology, Gram stain and biochemical properties. Automated Biometric Identification System (ABIS) online software for bacteria identification was referenced as a standard for morphological identification of bacteria. Morphological examination was carried out on Mannitol salt agar media used for isolation of the organism. Isolates were sub-cultured based on colony size, elevation, colour, shape and texture to obtain pure isolates. Gram stain and Biochemical test such as Coagulase, Catalase, Haemolysis and Sugar Fermentation tests were carried out to characterised and identify organism as described by Cheesbough, (2000).

2.8.1 Gram Staining

To make a smear, an isolate from primary culture was taken and placed on a dry, clean slide that was devoid of grease. The smear was heat fixed, then covered in crystal violet stain for a minute before being cleaned off with water. Lugol iodine stain was added and washed off with clean water after 1 minute of application. 70% ethanol (decolouriser) was added and allowed to stay for 3 seconds then washed off. Safranin was then added for 1 minute and washed off. The slide was air dried; oil immersion was placed and viewed under the microscope at 100X magnification (high resolution objectives lens) (Cheesbrough, 2000).

2.8.2 Catalase Test

A loopful of bacteria isolate was placed on a clean dry slide and a drop of 3% H_2O_2 was added and then observed for any reaction. Production of bubbles upon addition of hydrogen peroxide (H_2O_2) indicates a positive result (catalase positive) while absence of bubble indicates a negative result (Catalase negative) (Cheesbrough, 2000).

2.8.3 Oxidase Test

Few drops of Kovac's reagent were placed on a filter paper and a loopful of bacteria was added and then observed for any colouration. Production of violet purple colouration indicates a positive result (oxidase positive) while absence of purple colouration indicates a negative result (oxidase negative) (Cheesbrough, 2000).

2.8.4 Coagulase Test

A clean slide was obtained, and a drop of normal saline was applied to each end of the slide. The isolated colony was then picked up using a loop, and was emulsified to create two thick suspensions. One of the suspensions received a drop of blood plasma, which was gently mixed in before being checked for agglutination. To distinguish between the organism's potential granular appearance and genuine coagulase agglutination, no plasma was added to the second suspension. A positive result (coagulase positive) is indicated by any agglutination after the addition of plasma while no agglutination indicates a negative result (Coagulase negative) (Cheesbrough, 2000).

2.8.5 Sugar Fermentation Test

With a straight inoculation needle, an isolated colony of organisms from a pure culture was streaked onto the surface of Triple Sugar Iron Agar Slant (TSI). The medium was first stabbed through the middle to the bottom of the tube. The tube's cap was left loose, and it was incubated for 18 to 24 hours at 35 to 37 ^{o}c. The fermentation of glucose, lactose, and sucrose

is indicated by a colour shift of the slant and butt from red to yellow (an acid/acid reaction). No slant colour change, but a change from red to yellow in the butt implies solely glucose fermentation (an alkaline/acid reaction). No colour change of both slant and butt indicate absences of sugar fermentation result (An alkaline/alkaline reaction). Blackening of the medium indicates H_2S production. Bubbles or cracks in the medium indicate acid production (Cheesbrough, 2000).

2.8.6 Haemolysis Test

Isolates from pure culture were inoculated onto blood agar plate and incubated in inverted position at 35-37^{o}C for 24 hours. Plate was then observed for any colouration around colonies. Yellow colouration around colonies indicates complete haemolysis (β-haemolysis), green colouration indicates partial haemolysis (α-haemolysis) and no colouration around colonies indicates no haemolysis (ɣ-haemolysis) (Cheesbrough, 2000).

2.9 Preservation of Isolates

Isolates identified to be *Staphylococcus aureus* were preserved in 10% glycerol in bijou bottles which were refrigerated at -80^{O}C. 10% glycerol was prepared by diluting 10ml of glycerol with 90ml of distilled water and autoclaved at 121^{o}C for 15minutes. Suspension was allowed to cool and a sterile inoculating loop was used to transfer colonies of isolates from pure culture into the suspension until it becomes cloudy and then refrigerated at -80^{O}C (Missiakas and Schneewind, 2013).

2.10 Data Analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) software. Descriptive statistics for tabulation and graphical display were used to summarize the data. The measure of association was determined using chi-square test. Analysis of variance (ANOVA) was used to determine whether there was a significant relationship ($p \leq 0.05$) between variables and *Staphylococcus aureus* prevalence.

3.0 RESULTS

3.1 Distribution of Participants

A total of 103 women which include 55 (53.4%) non-pregnant women and 48 (46.6%) six weeks postpartum women as shown in Figure 1 were included in the present study. The highest numbers of participant were within 21-25 age group while the lowest were within 15-20 age group. Non-pregnant women participated more in the study than postpartum women (Table 1).

3.2 Prevalence of *Staphylococcus aureus* among Study Population

Out of a total of 103 women sample tested, 25 (24.3%) were found positive for *S. aureus* which included 20 (36.4%) non-pregnant women and 5 (10.4%) Postpartum women samples as shown in figure 2.

Among non-pregnant women population, the highest prevalence of 18.2% was recorded within 21-25 age group whereas lowest prevalence rate of 1.8% was observed in each 31-35 age group and >35 age group. Among postpartum population, the highest prevalence rate of 4.2% was found in each 31-35 age group and >35 age group while none of the sample tested found positive in 15-20 and 26-30 age group. The details are depicted in Figure 3. The analysis of the questionnaires returned is presented in Table 1-4.

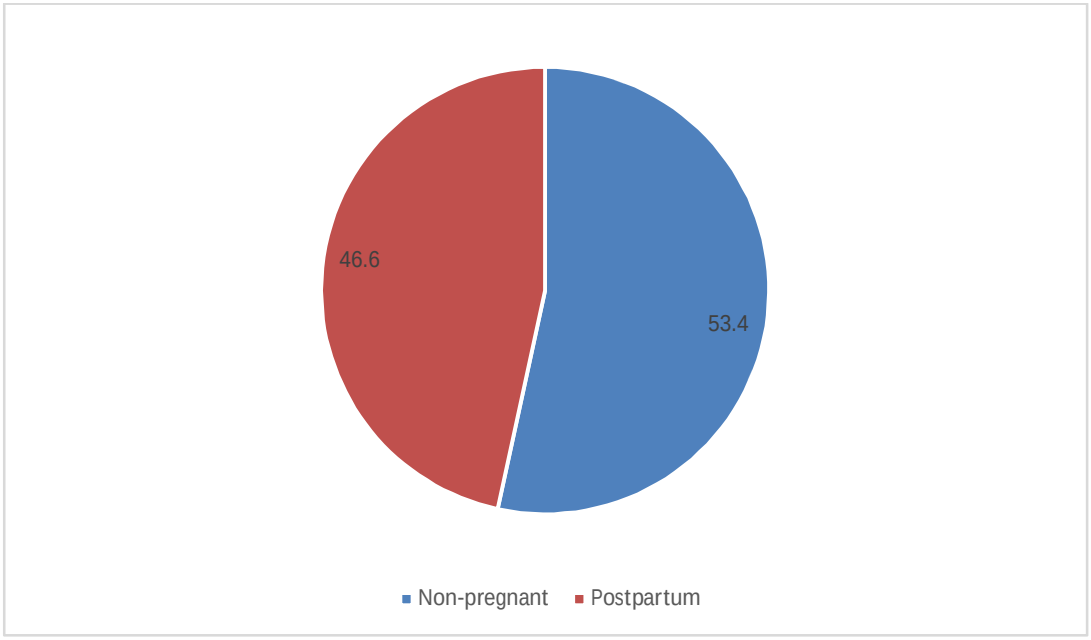


Figure 1: Number of non-pregnant and postpartum women involved in the study (%)

Table 1: Age group of Non-pregnant and postpartum women studied

Age Group (In Years)	Non-pregnant n (%)	Postpartum n (%)	Overall n (%)
15-20	13 (23.4)	0 (0)	13 (12.6)
21-25	31 (56.4)	4 (8.3)	35 (34.0)
26-30	8 (14.5)	10 (20.8)	18 (17.5)
31-35	2 (3.6)	17 (35.4)	19 (18.4)
≥ 36	1 (1.8)	17 (35.4)	18 (17.5)

Table 2: Marital status of Non-pregnant and postpartum women studied

Marital status	**Non-pregnant**	**Postpartum**
Single	52 (94.5)	3 (6.25)
Married	3 (12)	45 (93.8)
Separated	-	-

Table 3: Number of sexual partners of Non-pregnant and postpartum women studied

Number of Sexual partners	**Non-pregnant**	**Postpartum**
None	8 (14.5)	-
1	39 (70.9)	48 (100)
2	4 (7.3)	-
Multiple	1 (1.8)	-
Not applicable	3 (5.5)	-

Table 4: Other data of Non-pregnant and postpartum women studied

Variables	**Non- pregnant**	**Postpartum**	**P- value**

	Yes n (%)	No n (%)	Not applicable n (%)	Yes n (%)	No n (%)	Not applicable n (%)	
Knowledge	44 (80)	11 (20)	-	38 (79.2)	10 (20.8)	-	.236
Symptomatic Cases	33 (60)	15 (27.2)	7 (12.7)	11 (22.9)	32 (66.7)	5 (10.4)	.883
History of Previous Infection	19 (34.5)	35 (63.6)	1 (1.8)	10 (20.8)	37 (77)	1 (2.8)	.133
Treatment	18 (32.7)	1 (1.8)	36 (65.5)	8 (16.7)	-	40 (83.3)	.052*
Regular Screening	3 (5.5)	52 (94.5)	-	2 (4.2)	46 (95.8)	-	

*Correlation is significant at the .05 level

Table 5: Cultural and Biochemical Characteristics of Bacterial Isolates

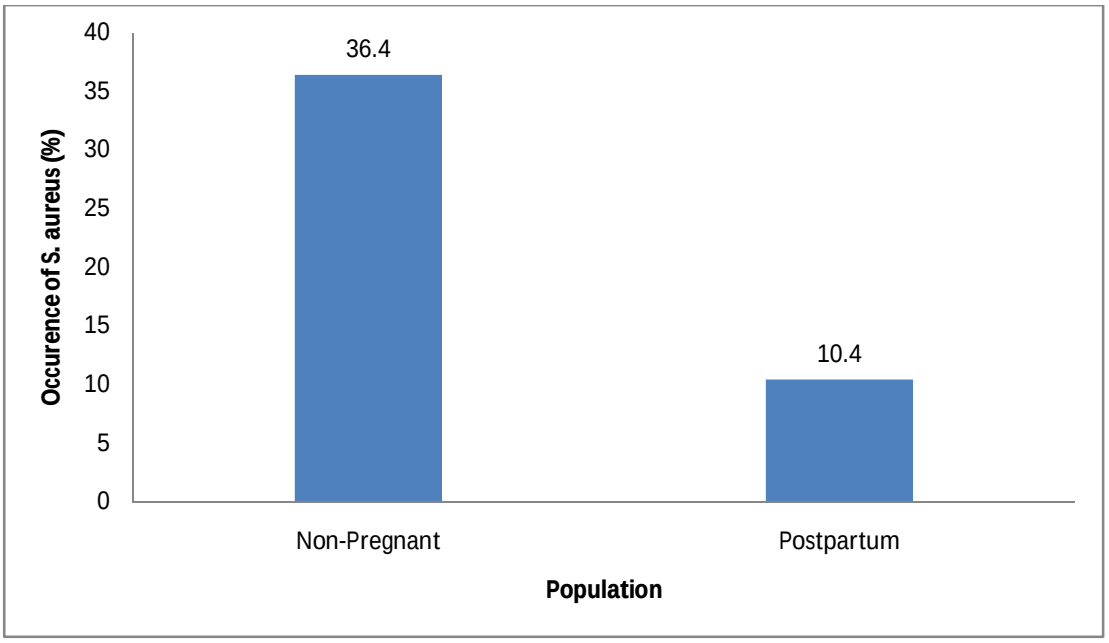
Isolates	Shape	Gram	Appearance	Catalase	Coagulase	Glucose	Mannitol	Sucrose	Lactose	Hemolysis	Suspected Organism
P-1	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
P-9	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
P-14	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
P-45	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
P-48	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-3	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-4	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-5	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-6	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-7	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*

NP-10	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-11	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-12	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-13	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-14	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-15	Cocci	+	Clustered	+	+	+	+	+	+	β	*S. aureus*
NP-28	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-29	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-30	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-40	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*

NP-48	Cocci	+	Clustered	+	+	+	+	+	+	β	*S. aureus*
NP-49	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-52	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-52	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*
NP-54	Cocci	+	Clustered	+	+	+	+	+	+	ɣ	*S. aureus*

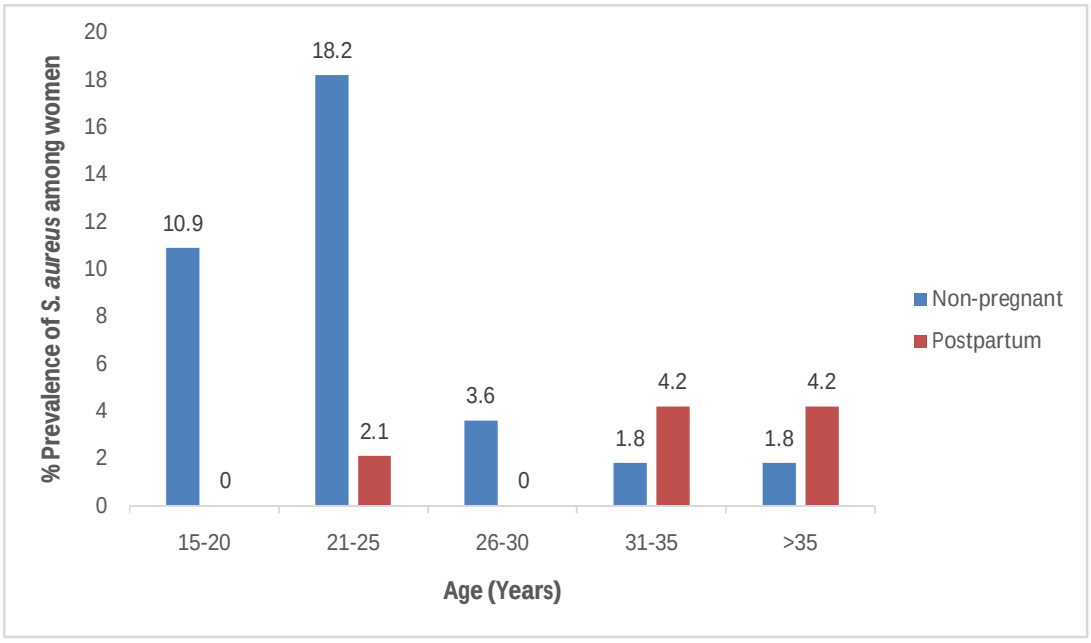
Morphology for *Staphylococcus aureus*: smooth, golden yellow, slightly raised

Key: + positive; - Negative, NP- Non-pregnant, P- Pregnant, β- Beta, ɣ- Gamma



P- Value = .002 (*P*≤ .05)

Figure 2: Frequency of *S. aureus* in Non-pregnant and Postpartum women (%) studied



P- Value = .040 (*P*≤ .05)

Figure 3: Prevalence of *S. aureus* in Non-pregnant and Postpartum women according to age group

4.0 DISCUSSION

Reproductive tract infection in women is a major cause of concern to public health as cases of infertility, pregnancy complications, newborn abnormalities and increased spread of sexually transmitted diseases (STD's) has often been associated with it (Robert *et al.,* 2005; Donati *et al.,* 2010). The analyses identify the prevalence of *Staphylococcus aureus* in women of the study area as 24.3%. This observation is similar to 20% reported by Maidawa *et al.,* (2021) from some hospitals within Kaduna metropolis, Northwest of Nigeria but much lower than that (57.3%) recorded by Mahdieh *et al.,* (2014) from Qom City in Iran and 58.7% reported in female from Yenagoa, Bayelsa in Nigeria (Adebola and Godwin, 2012). This demonstrates that results from various nations and cities exhibit significant geographical diversity which can be attributed to factors such as level of knowledge of the infection, cultural practices (Marriage type), sexual behaviour and hygienic standard of the people amongst others (Kurewa *et al.,* 2010). The high level of knowledge of the infection (77.6%) seen in the research area can also be a contributing factor to the low prevalence of *Staphylococcus aureus* observed when compared to other previous findings (Mahdieh *et al.,* 2014; Adebola and Godwin, 2012).

Non-pregnant women show a higher rate of *Staphylococcus aureus* colonisation than postpartum women as revealed by the study. Similarly, a high isolation rate of *Staphylococcus aureus* in Non-pregnant women than in pregnant women attending a tertiary Hospital in Port Harcourt have also been reported (Stanley *et al.,* 2013). There was significant difference in the prevalence of *Staphylococcus aureus* between Non-pregnant women and postpartum women (P= .002). This difference might be as a result of routine prophylactic antibiotics administered after delivery which reduces maternal infectious morbidity (Bonet *et al.,* 2017).

The observed variations in isolation rates across the different age groups were statistically significant (P= .040). A high occurrence of *S. aureus* was seen in women within 21-25 age group. This is in agreement with the findings of Adebola and Godwin, (2012) who recorded high prevalence of *S. aureus* in women within 21-30 years of age. One might infer that these are the age ranges for both sexual activity and child bearing.

Data collated from the study also revealed that majority of women in this region rarely go for reproductive tract screening. This might have contributed to the high prevalence of the infection. The correlation of *Staphylococcus aureus* presence and treatment of previous reproductive tract infections disclosed by the study shows an indiscriminate use of antibiotics

in this region arising from self medication which is frequently linked to insufficient dosage and noncompliance with therapy, as well as the accessibility of antibiotics to consumers over the counter with or without a prescription (James *et al.,* 2017). Sexual involvement with more than one individual has long been established as a predisposing factor for reproductive tract infections (Oliveira *et al.,* 2007)). The study shows that 4.9% of the women precisely non-pregnant women had more than one sexual partners and majority of the singles are actively involved in sexual activity with partner(s) whose sexual faithfulness is questionable.

The present study shows no significant correlation between symptomatic cases and prevalence of *Staphylococcus aureus.* However, a high percentage of women that tested positive for the infection were asymptomatic as at the time of sample collection. This suggests that healthy individual can serve as a carrier and disseminator of this infection (Ehinmidu, 2003). Therefore, the need for regular *Staphylococcal* infection screening is of necessity.

LIMITATION OF THE STUDY

Not including pregnant women in the study may lead to under estimation of the overall prevalence rate of *Staphylococcus aureus* among female patients attending Rivers State University Teaching Hospital.

CONCLUSION

In view of the outcome of the study, it can be concluded that females within reproductive ages are most vulnerable to *Staphylococcal* infection. The correlation of *Staphylococcus aureus* prevalence and treatment of previous reproductive tract infection seen in the study suggest that regular *Staphylococcal* screening of women should be greatly encouraged and Staphylococcal antibiotic resistance surveillance should be reviewed.

Ethical Approval and Consent

From the hospital's appropriate board, ethical permission was obtained. Consent of participant was sorted by signing in consent form.

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