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Occurrence of *Staphylococcus* associated with Urinary Tract Infections among women attending Ibrahim Badamasi Babangida (IBB) specialist hospital, Minna, Nigeria.

[1]Baba John, *Mabekoje Oladele O,[1] Haruna Aminat[1], Mohammed A. Saba[2], Dauda Danasabe[1], Muhammad I. Legbo[1], Sani A. Rabiu[1] and Jibril F. Ladidi[1].

[1]Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Nigeria.
[2]Department of Biological Sciences, Niger state Polytechnic, Zungeru, Nigeria.

Abstract

Background: Urinary tract infection (UTI) refers to any part of urinary system infection caused by the presence and growth of microorganisms anywhere in the urinary tract. The infections are commonly found in women. *Staphylococcus saprophyticus* may infect the urinary tract.

Methods: Isolation of *Staphylococcus* spp was carried out using the streak plate method on Mannitol salt and Blood agar. Pure isolates were subjected to different biochemical tests for identification. Kirby bauer method was used to determine the antibiotic susceptibility profile of the isolates.

Results: *S. saprophyticus* accounted for 27(55.1%) of the isolates, *S.aureus* accounted for 14 (28.6%) of the isolates, and *S. epidermidis* accounted for 8 (10.2%) of the isolates. Most of the isolates were susceptible to Levofloxacin and Rifampin, while Erythromycin, Clarithromycin, Streptromycin and Amoxicillin were resisted by the isolates of the *Staphylococcus* species. *S. saprophyticus* isolates were susceptible to Levofloxacin (55.6%) and least susceptible to Norfloxacin (11.1%). *S. epidermidis* were more susceptible to Levofloxacin (75.0%), and resistant to Ampicillin (0.0%), while *S. aureus* was most susceptible to Levofloxacin (78.6%) and least to Ampicillin and erythromycin (14.3%). Age, occupation, marital status and area of domicile of the patients were found to be associated with the occurrence of *Staphylococcus* species in the urine of patients ($P<0.5$). Educational status indicated no association with the incidence of *Staphylococcus* species in the urine of patients ($P>0.5$).

Conclusion: *S. saprophyticus* was found as the most predominant *Staphylococcus* species in the urine of women attending IBB specialist hospital Minna, Niger state, Nigeria.

Keywords: *Staphylococcus* species; Urinary tract infections; Women

Introduction

Urinary tract infections (UTIs) refer to urinary tract inflammatory disorders, resulting from abnormal pathogens growth (Amali *et al.,* 2009; Prakash *et al.*, 2013). It can simply refer to the condition women may encounter during their lifespan, where young and pregnant women encounter the highest prevalence of urinary tract infections. UTI may be community-acquired or hospital-acquired. Community-acquired infection is said to be the second most encountered infection of the microbes in our community setting (Lacovelli *et al.,* 2014). Community-acquired urinary tract infections (CA-UTIs) are defined as urinary system infections in one's life; it takes place in the community setting or hospital environment within a period of less than 2days of admission. In terms of lower abdominal pain (LAP), dysuria and fever, urinary tract infection is known to cause short-term morbidity and also may result in permanent kidney scarring (Camacho *et al.,* 2004). UTIs may be chronic, asymptomatic, acute and uncomplicated or complicated, clinical manifestation varies on the aetiology of the organism, the immune response of the patient, urinary tract portion involved.

***Corresponding author**: delemabekoje70@gmail.com, Tel: 08057119456.

Both symptomatic and asymptomatic UTIs, seriously pose a threat to public health care, therefore leading to the reduction of life quality and resulting in absenteeism from work (Olowe *et al.,* 2015).

The age and location of urinary tract infection determine the symptoms of UTIs, viz itching, fever, LAP, genital and suprapubic pain, burning sensation while urinating, ulcers in the genital area and formation of blisters and pyuria (Amali *et al.,* 2009). Several factors which include, race, gender, circumcision, age (Dias *et al.,* 2010), HIV (Banu *et al*., 2013), genitourinary tract abnormalities, urinary catheter, diabetes (Mladenovic *et al.,* 2015), elderly pregnancy, infants (Nelson *et al.,* 2015), and hospitalization status (Adukauskiene *et al.,* 2006) bear recurrent significant risk.

UTIs are caused majorly by the presence of bacteria in urine, other microorganisms as etiological agents may however not be ruled out. UTIs are commonly found in women more than men due to the anatomical differences between them; distance between their urethra and anus, and urinary tract shortness (Ehlers *et al.,* 2020). *Staphylococcus saprophyticus* and *Staphylococcus aureus* cause an estimated 5 - 15% of UTIs frequently in younger women (Micheal *et al*., 2007).

The desire to establish more facts on the different species of *Staphylococcus* as etiological agents of UTIs necessitated this research. The research is aimed at assessing the prevalence of UTIs among the women in our community that attends Ibrahim Badamasi Babangida University specialist hospital, Minna, Nigeria, with the intention of proffering solution that will ameliorate this disease occurrence among women in the community; by way of evaluation of likely socio-demographic or risk factors that could be associated with the presence of *Staphylococcus* species in the urine of the patients.

Materials and Methods

Study area

The study was conducted at IBB Specialist Hospital, Minna. The hospital is located in Chanchaga Local Government Area of Niger State, Nigeria. Its headquarters is in the state capital of Minna, which occupies much of the Local Government Area. It has an area of 72km^2 and a population of 201,429 at the 2006 census which has coordinates of 9°36'50"N6°33'25" E.

Fig 1: Map indicating Local Government areas of Niger state.

Inclusion criteria
Only women diagnosed with urinary tract infections were included in the study.

Exclusion criteria
Patients not suffering from urinary tract infections were excluded from the study.

Sample collection
A total of hundred (100) urine samples were collected using a sterile container from female individuals with different types of urinary tract infections (sterile gloves were used to avoid cross-contamination). The sterile containers containing the urine were transported to the Laboratory of Microbiology department of Ibrahim Badamasi Babangida University, Lapai within 24 hours in a non-metal container maintained and refrigerated by surrounding the container with an ice park, where the analysis was carried out. Questionnaires were administered to the patients at the point of sample collection to determine the age, marital status, occupation, educational qualification, and area of domicile of the patients as the likely socio-demographic factors that could be associated with the presence of Staphylococcus species in the urine of the patients.

Microscopic examination of urine
Freshly collected urine samples were centrifuged and the deposits were microscopically examined for the presence of pus cells, red blood cells, epithelia cells, bacteria cells, and cast and yeast cells. Ten (10) ml of a mixed urine sample was aseptically transferred in a labelled test tube; centrifuged for 3-5 minutes, and the supernatant was poured into another container. The sediment was remixed by tapping the bottom of the bottle; a drop of the well-mixed sediment was then transferred to a slide and then covered

with a cover slide. The prepared slide was mounted on the microscope and then examined using ×10 and ×40 for a clearer view (Cheesbrough, 2006)

Isolation of *Staphylococcus* spp from urine

Culturing of the urine sample: Two different media were used to culture the urine specimen i.e. Blood agar and Mannitol salt agar. A sterile inoculating loop was used to inoculate the media with the urine specimen, which was properly mixed by rotating it before inoculation. The urine sample was then streaked on the surface of the solidified agar media using a wire loop aseptically. The plates were then incubated aerobically at 37°C for 24 hours. Distinct colonies were sub-cultured on other sets of sterile Blood and Mannitol salt agar plates to obtain pure isolates and then incubated for another 24 hours at 37°C. The plates were observed for cultural characteristics of *Staphylococcus* species (Cheesbrough, 2006)

Characterization and Identification of *Staphylococcus* species

The bacterial isolates were characterized based on their colonial morphology, cellular morphology and biochemical characteristics. Microscopic examination of individual cells from distinct colonies was done through the Gram stain technique. Isolates were subjected to a series of biochemical tests, such as Catalase, Coagulase, Oxidase, Indole, Methyl red, Vogues Proskauer and Sugar fermentation tests for further identification (Cheesbrough, 2006).

Antibiotic Susceptibility test

Sterile Mueller Hinton agar was prepared as instructed by the manufacturer. The media was dispensed into Petri dishes and allowed to solidify, the inocula were prepared in the test tubes in conformity to 0.5 McFarland standard, and the test isolates were inoculated on the Mueller Hinton agar using a sterile swab stick, after which the antibiotic discs were placed onto the inoculated plates using a sterile forceps. The following antibiotic discs were used: gentamicin (CN)10µg, amoxicillin (AMX) 30µg, streptomycin(S) 30µg, clarithromycin(CH) 30µg, ampicillin (APX) 30µg, ciprofloxacin (CPX) 10µg, norfloxacin (NB) 10µg, rifampin(RD) 20µg, erythromycin(E) 30µg, levofloxacin (LEV) 20µg. The plates were incubated at 37°C for 24 hours. The plates were then read and the zone of inhibition was measured to the nearest mm (Cheesbrough, 2006).

Novobiocin Susceptibility of Coagulase-negative staphylococci.

Coagulase-negative staphylococci specie isolates were subjected to Novobiocin antibiotics to differentiate between *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*. Suspension of the test isolates was prepared in tryptic soy broth to a McFarland 0.5 standard. A sterile swab was immersed into the suspension and rotated against the side of the tube above the fluid level to remove the excess inocula. The swab was then used to inoculate a Mueller Hinton agar plate by streaking the swab over the entire agar surface. Using sterile forceps, novobiocin antibiotic discs were aseptically applied to the surface of each inoculated plate. The discs were pressed gently with sterile forceps to ensure contact with the agar surface. The plates were incubated aerobically for 24 hours at 37°C. The diameter of the zones of inhibition was measured to the nearest millimetre with a metric ruler(Murray, 2007).

Statistical analysis

Statistical analysis was carried out to determine the association between socio-demographic factors and the occurrence of *Staphylococcus* species in the urine of patients with UTI. Chi square statistical tool was used to determine the association between socio-demographic factors and the occurrence of *Staphylococcus* species in the urine of patients with UTI. The age, occupation, area of domicile, marital status, and educational qualification were the socio demographic factors considered.

Results

Staphylococcus saprophyticus predominate among the bacteria isolated from the urine of the patients that consented to participate in the study with 27(55.1%) isolates, while *Staphylococcus epidermidis* occurred least in the urine of the patient with 8(16.3%) isolates.

Table 1: Occurrence of staphylococcus species isolated from urine among women attending IBB Specialist Hospital, Minna

Isolates	Number of isolates	Percentage occurrence (%)
Staphylococcusaureus	14	28.6
Staphylococcus saprophyticus	27	55.1
Staphylococcus epidermidis	8	16.3
Total	49	100.0

Most of the isolates were susceptible to levofloxacin and rifampin, while erythromycin, clarithromycin, streptomycin and amoxicillin were resisted by the isolates of the *Staphylococcus* species. *S. saprophyticus* isolates were more susceptible to levofloxacin (55.6%) and least susceptible to norfloxacin (11.1%). *S. aureus* was more susceptible to levofloxacin (78.6%) and least to ampicillin (14.3%) and erythromycin (14.3%), *S. epidermidis* were more susceptible to levofloxacin (75.0%) and resistance to ampicillin (0.00%).

Table 2: Percentage Susceptibility of the isolates to different antibiotics

Antibiotics	***S.saprophyticus*** **n= 27**	***S.aureus*** **n=14**	***S.epidermidis*** **n=8**
Levofloxacin	55.6(44.4)	78.6(21.4)	75.0(25.0)
Gentamicin	29.6(70.4)	35.7(64.3)	50.0(50.0)
Ampicillin	37.0(63.0)	14.3(85.7)	100.0(0.0)
Rifampin	48.1(51.9)	64.3(35.7)	50.0(50.0)
Amoxicillin	25.9(74.1)	35.7(64.3)	25.0(75.0)
Streptomycin	29.6(70.4)	35.7(64.3)	25.0(75.0)
Norfloxacin	11.1(88.9)	28.6(71.4)	12.5(87.5)
Clarithromycin	22.2(77.8)	28.6(71.4)	25.0(75.0)
Ciprofloxacin	37.0(63.0)	42.9(57.1)	50.0(50.0)
Erythromycin	33.3(66.7)	14.3(85.7)	25.0(75.0)

KEY: n = Number of isolates
NUMBERS OUTSIDE BRACKET = Susceptibility percentage
NUMBERS INSIDE BRACKET = Resistance percentage

The results of the Novobiocin susceptibility testing of the coagulase-negative staphylococci to differentiate between the isolates of *Staphylococcus saprophyticus* from *Staphylococcus epidermises* as indicated in the pie chart below, clearly implies that *Staphylococcus saprophyticus* isolates were resistant to the novobiocin antibiotics (25% susceptible) as compared to the isolates of *Staphylococcus epidermidis* that were susceptible (75% susceptible) to the antibiotics.

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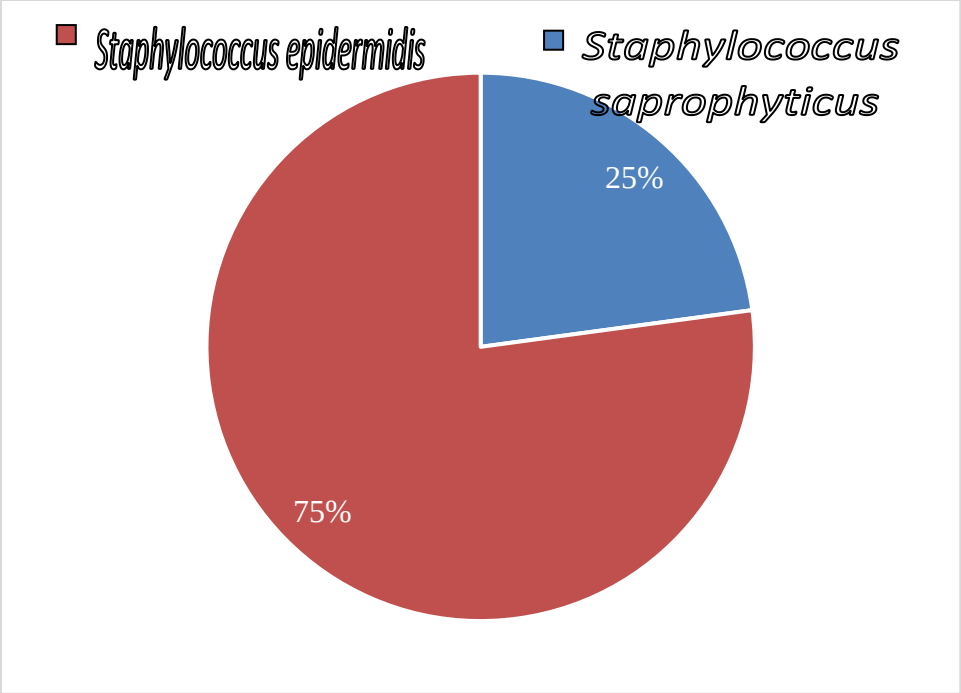


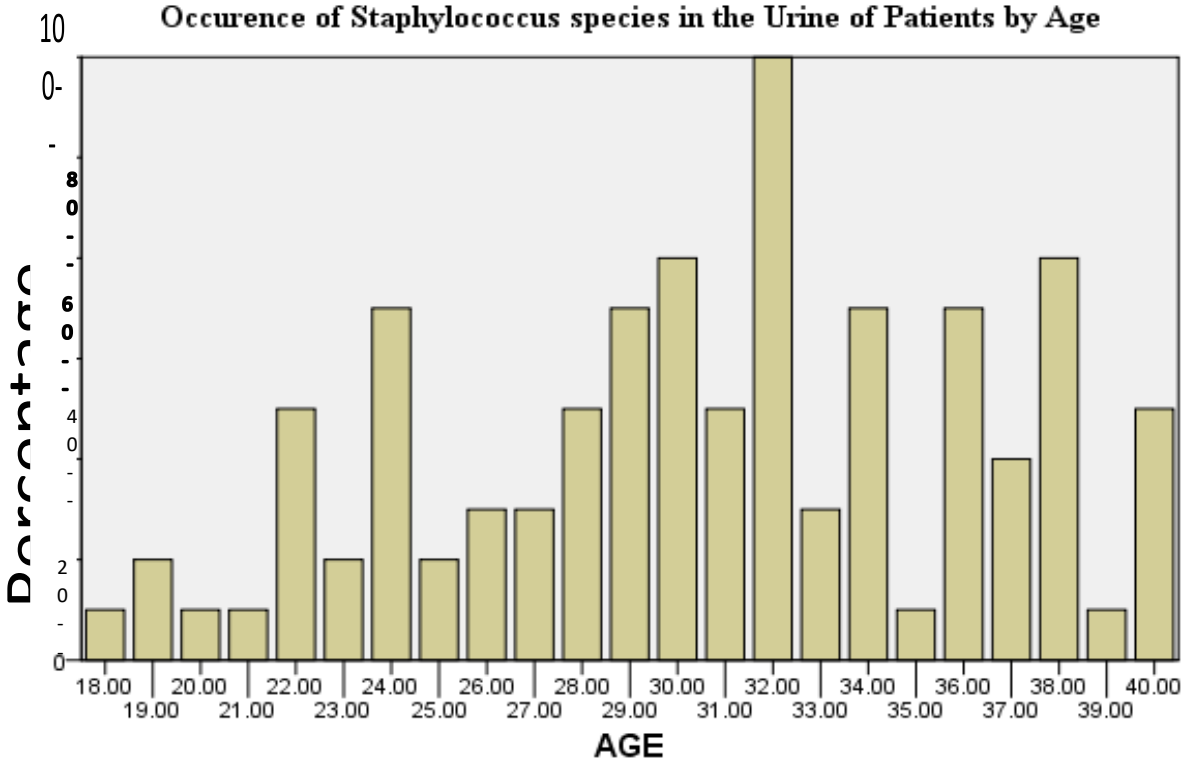
Figure1: Percentage Susceptibility of Coagulase-negative staphylococci to Novobiocin antibiotics.

Statistical analysis using CHI square was used to access the relationship between the socio-demographic factors and the occurrence of *Staphylococcus* species in the urine of patients among women attending IBB Specialist Hospital in Minna, Niger State. The socio–demographic factors that were considered are; the age of the respondents, marital status, educational status, area of domicile and occupation of the patients.

Occurrence of *Staphylococcus* species in the urine of patients by age.

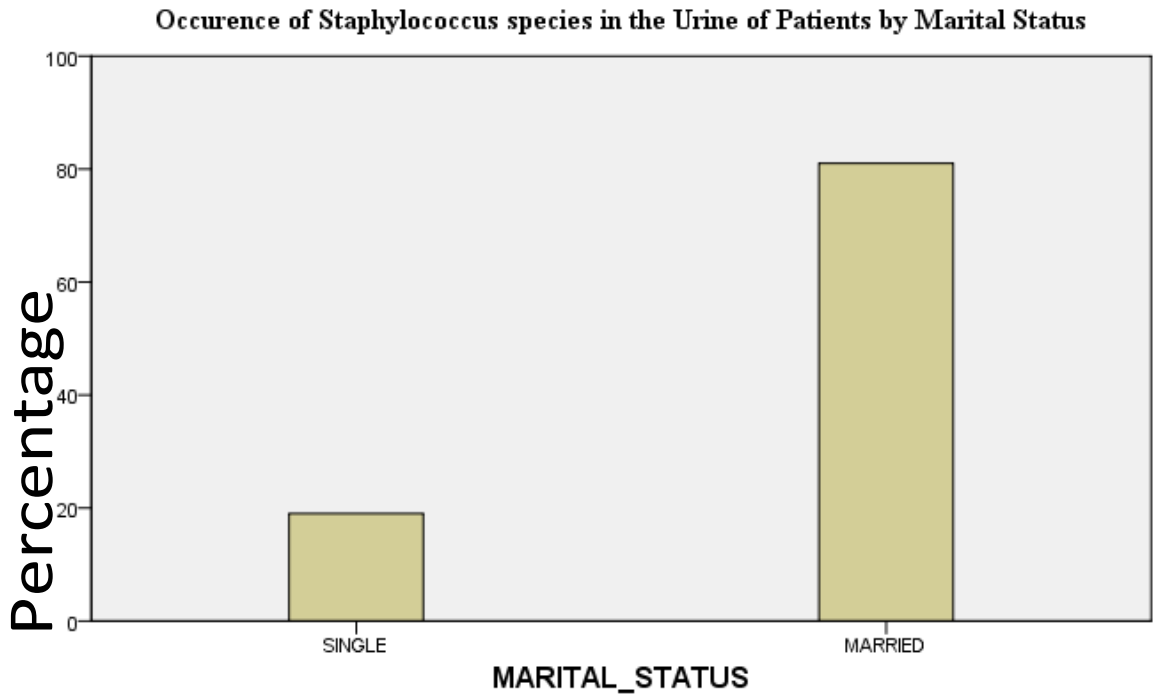
The analysis indicated that there is an association between the age of the patients attending IBB Specialist Hospital and the occurrence of *Staphylococcus* species in the urine of the patients($P < 0.05$). The implication of this is that age is a determinant of the occurrence of *Staphylococcus* species in the urine of

patients. This is indicated in the graph below



(P <0.0.5)
Figure 2: Occurrence of *Staphylococcus* species in the urine of patients by age.

Occurrence of *Staphylococcus* species in the urine of patients by Marital status.
The statistical analysis indicated that the marital status of the respondents in the study area is a determinant to the occurrence of *Staphylococcus* species in their urine (P<0.05). The results also indicated that married individuals are more prone to the occurrence of *Staphylococcus* species in their urine. This is indicated in the graph below.



(P <0.05)
Figure.3: Occurrence of *Staphylococcus* species in the urine of patients by marital status.

Occurrence of *Staphylococcus* species in the urine of patients by Educational status.
Educational status of the respondents was considered not to be a factor to the occurrence of *Staphylococcus* species in the urine of patients. The analysis ofthe result showed that there is no association between the educational status and the occurrence of *Staphylococcus* species in the urine of the patients (P>0.05).

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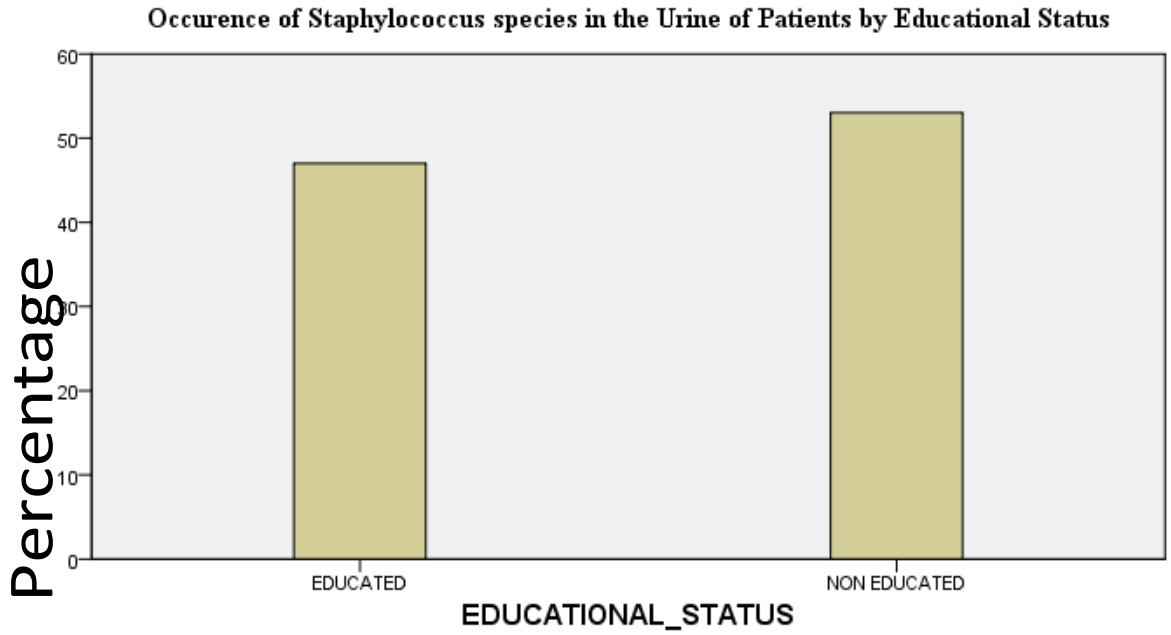
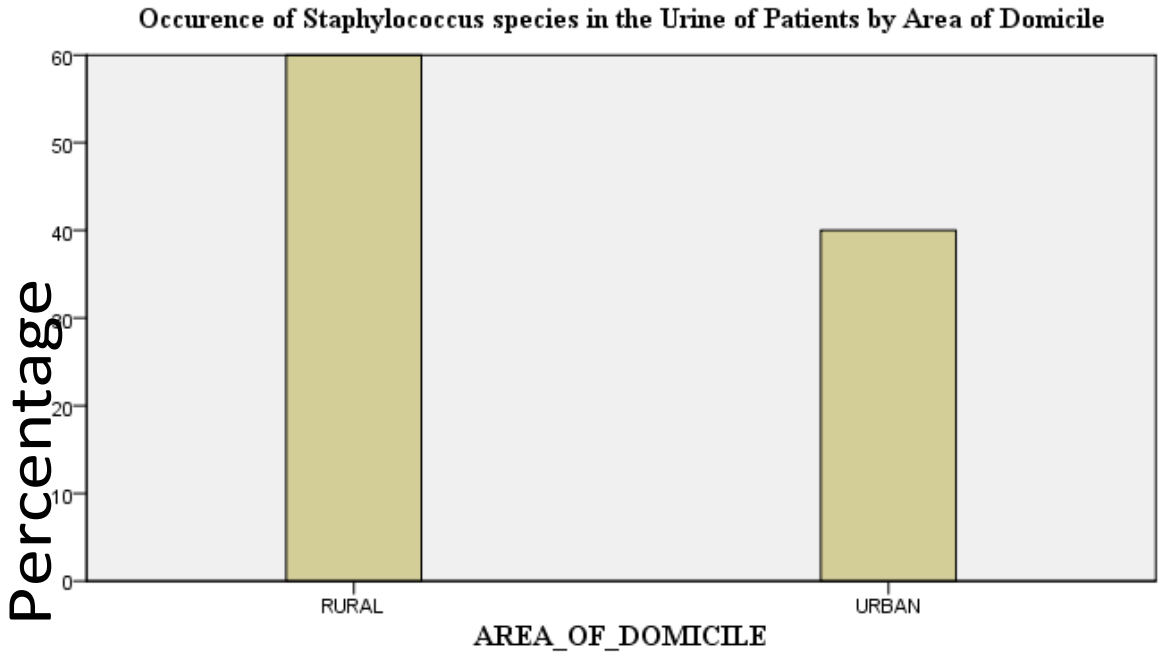


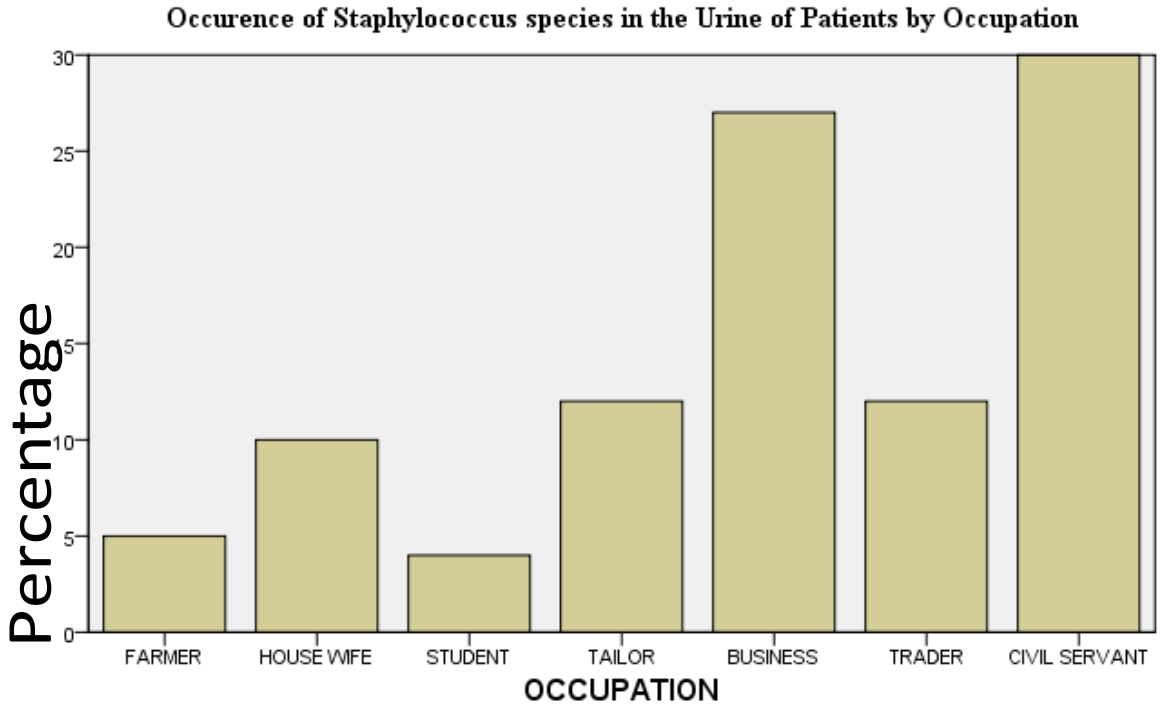
(P > 0.05)
Figure 4: Occurrence of *Staphylococcus* species in the urine of patients by Educational status.

Occurrence of *Staphylococcus* species in the urine of patients by area of domicile.
The analysis of the result shows that there is an association between the area of domicile of the sampled respondents and the occurrence of *Staphylococcus* species in their urine (P<0.05). Women who lived in rural areas are more prone to the bacterial species in their urine. This is shown in the graph below



(P < 0.05)
Figure 5 : Occurrence of *Staphylococcus* species in the urine of patients by area of domicile.

Occurrence of Staphylococcus species in the urine of patients by Occupation.
The analysis indicated that there is association between occupation of the respondents in the study area and the occurrence of *Staphylococcus* species in their urine. (P < 0.05). The implication of this is that occupation is a factor to the occurrence of the *Staphylococcus* species in the urine of the patients. This is indicated in the graph below



(P< 0.05)

Figure 6: Occurrence of Staphylococcus species in the urine of patients by Occupation.

Discussion

Three species of *Staphylococcus* were recovered as possible etiological agents for UTI in this study. Only two among these species were reported to cause UTIs (*Staphylococcus aureus* and *Staphylococcus saprophyticus*), *Staphylococcus epidermidis* is assumed to have been isolated in the urine as a contaminant (Dobkin *et al*., 2011). A previous study has linked the increase in *Staphylococcal* UTIs to an increase in the use of instruments such as bladder catheters (Iregbu *et al*., 2013).

In this study, the incidence of *Staphylococcus* species associated with UTIs among women was encountered among those that were within the age bracket of 21-30, the women within this group of age are sexually active. Sexual activity promotes a great chance and transmission of UTI among women (Nicolle, 2012). *S. sprophyticus* was the most prevalent pathogen and *S.epidermidis was* the least. *S. aureus* was reported as the predominant uropathogenic isolated from patients with UTI signs and symptoms (Henry *et al*., 2011), which is not in agreement with the findings of this study. *S.aureus* was the second most isolated pathogen in this study which did not agree with the findings of Martin *et al*. (2019), but similar to the findings of Ekwealor *et al*. (2016) which reported high rates of *S.aureus*. *S.saprophyticus* has been reported as the most frequently cultured uropathogenic (McCormick *et al*., 2008). Urethral, rectal or vaginal colonization with *S.saprophyticus* has been associated with UTI (Raze*tal*., 2005). The prevalence of *S.aureus* observed in the urine in a study conducted by Nworie *et al*. (2013) might be a result of poor hygiene.

Socio-demographic factors of age, occupation, marital status and area of domicile of the patients were found to be associated with the occurrence of *Staphylococcus* species in the urine of patients in this research. Conway *et al.* (2007) and Dias *et al.* (2010) reported similar findings in their separate studies.

Most of the isolates were susceptible to Levofloxacin and Rifampin, while Erythromycin, Clarithromycin, Streptromycin and Amoxicillin were resisted by the isolates of the *Staphylococcus* species which could be a result of irrational use of antibiotics and self-medication by patients to treat all kinds of infections; this is in agreement with the study of Ejikeugwu *et al*. (2013). *S.saprophyticus* isolates were susceptible to Levofloxacin and least susceptible to Norfloxacin. *S. epidermidis* were more susceptible to Levofloxacin, and showed total resistance to Ampicillin, this is in agreement with the findings of Cafiso *et al.* (2001) who reported the potency of Levofloxacin against *S. epidermidis*. *S.aureus* were most susceptible to Levofloxacin and resistant to Ampicillin and Erythromycin.

Muhammad *et al.* (2013) in a similar study reported the susceptibility of *S. aureus* to Levofloxacin. Nwankwo & Nasiru (2011) also reported *S. aureus* susceptibility to Levofloxacin and resistance to Streptomycin and Erythromycin.

Novobiocin susceptibility testing results in this study clearly indicated that *S.saprophyticus* is resistant to Novobiocin. This may be due to the fact that *S. saprophyticus* is innately resistant to the antibiotic (Murray *et al.,* 2007). Murray *et al*. (2007) also reported coagulase-negative staphylococci resistance to Novobiocin including *S. saprophyticus.* The susceptibility of *S. epidermidis* to Novobiocin in this study could be traced to the fact that the antibiotic is an aminocoumarin antibiotic with antibacterial properties (Murray *et al.,* 2007). Adriano *et al*. (2012) reported *S. saprophyticus* to be generally identified based on Novobiocin resistance. Kahlmeter (2003) corroborated this; that identification of *S. saprophyticus* is through its resistance to Novobiocin antibiotics.

The process of recruiting women to participate in the research was however difficult, this is so because a majority of them were not willing to consent for their sample to be collected; which in turn limited the sample size to One hundred (100).

Conclusion

This study revealed that the incidence of *Staphylococcus* species in the urine of women attending IBB specialist hospital Minna is high when compared to the previous findings, with *S.saprophyticus* found as the most predominant *Staphylococcus* species. Age, occupation, area of domicile and marital status were the socio-demographic factors found to be associated with the occurrence of *Staphylococcus* species in the urine of the patients, according to the statistical analysis conducted. *Staphylococcus* species isolates were mostly susceptible to Levofloxacin and Rifampin.

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