Incidence of *Neisseria Gonorrhoeae* among Patients attending Selected Hospitals in Minna, Nigeria

Baba J.[1*] Mabekoje O. O.[1] Sanda I.[1] Majiya H.[1] Baba U. A.[4] Chock J. J.[2] Abdul-Rahaman A. A.[3] Abdullahi M.[5] Jibril F. L.[1] Dauda D.[1] and Sani A. R.[1]

1. Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Nigeria
2. Department of Medical Laboratory Sciences, Bingham University, Nasarawa State, Nigeria
3. Department of Microbiology, Federal University Lokoja, Nigeria
4. Ministry of Health, Niger State, Nigeria
5. Department of Biological Sciences, Niger State Polytechnic Zungeru

* Corresponding author: babajohn322@gmail.com

Abstract: Gonorrhoea is a sexually transmitted disease (STD) caused by infection with the *Neisseria gonorrhoeae* bacterium. This work was carried out from April to June, 2022 to determine the incidence of *Neisseria gonorrhoeae* among patients attending some selected Hospitals in Minna, Niger State. One hundred and twenty (120) samples collected, which includes, high vaginal swabs,81 (67.50%) , endo cervical swabs 16 (13.33%) from female patients and urethral swab, 23(19.17%) from male patients. The samples were cultured on chocolate agar modified with antibiotics. Isolation and identification of the organisms were done by standard bacteriological procedure. Antibiotic susceptibility profiles of the isolates were determined by Kirby-Bauer disc diffusion method on Muller Hinton agar. *Neisseria gonorrhoeae*was not isolated out of the 39 isolates obtained in the study as follows; *Escherichia coli* 13(33.3%), *Staphylococcus aureus* 12(30.8%), *Streptococcus* sp 3(7.7%) *Klebsiella* sp. 2(5.1%) and *Pseudomonas aeruginosa* 9(23.1%). The antibiotics susceptibility testing revealed *E. coli* were sensitive to ciprofloxacin, septrin, and pefloxacin. Apart from *E. coli,* other isolates sensitive to these antibiotics includes, *Streptococcus* sp., *Staphylococcus aureus* and *Klebsiella* sp. *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus* sp. were 100% resistant to amoxicillin, chloramphenicol, rifampin, augmentin and gentamicin. Twenty-three (23) (19.17%) positive cases of genitourinary tract infection were male, while female patients had 97(80.83%). During this research, middle aged patient from 25-30 (30.80%) and 20-24 (25.60%) were found to possess the highest prevalence of genitourinary tract infection. The findings of this study indicated that *E. coli* and *Staphylococcus aureus* were the most prevalent isolates responsible for genitourinary tract infection due to the possession of some virulent associated features which helps them to attach to the genital tract. Proper measures should be taken to prevent further spread of this infection most especially in women and children.

Key word: Genitourinary tract bacteria, *Neisseria gonorrhoeae*, patients, sexually transmitted disease.

INTRODUCTION

Neisseria gonorrhoeae (Gonococcus) is an organism that causes gonorrhea, a sexually transmitted infection (STI) that continues to be a global public health issue. The World Health Organization (WHO) research on clinical species of *N. gonorrhoeae* has identified species that are resistant to many antibiotics, demonstrating the imminent dangers of untreated gonococcus infections. The World Health Organization recommended eliminating this organism through treatment and preventing the spread of antimicrobial-resistant gonorrhea (WHO, 2016). With a global incidence of over 87 million new cases per year, uncontrolled transmission, and limitless treatment options in low-income countries and poor communities in advanced countries, untreated gonorrhea will lead to an increase in disease incidence and complications (Newman *et al.,* 2015; WHO, 2016; Carmona-Gutierrez *et al.*, 2016; Unema *et al*., 2016). The *N. gonorrhoeae* mainly colonizes the genital mucosa and also capable of colonizing the ocular, anal mucosa and nasopharyngeal (Danby *et al*., 2016). Pathogenicity is frequently caused by the stimulation of innate immune responses at the site of colonization, as the infection does not produce direct potent exotoxins. Untreated and aggravating female genital tract infection can lead to pelvic inflammatory disease (PID), ectopic

pregnancy, and infertility (Little, 2006). Neonatal blindness can also occur during childbirth as a result of maternal transmission, and if the infection is not treated, it can lead to infectious endocarditis and arthritis (Quillin and Seifert, 2018). *Neisseria gonorrhoeae* is from the genus *Neisseria*, only two of the 11 species colonizes humans such as *Neisseria gonorrhoeae* and *Neisseria meningitidis* (Meningococcus) which mainly causes bacterial Meningitis (Hoffman and Weber, 2009). Furthermore, some groups of 8 nonpathogenic commensal of *Neisseria* sp. are capable of colonizing the human oropharyngeal and nasal flora (Marri *et al.*, 2010). Other species of *Neisseria* are capable of inhibiting a range of non-human host and non-mammalian host, these are non-human primates, dogs, insects, avian species and herbivorous mammals and so on (Liu *et al.*, 2015). It is reported that *Neisseria gonorrhoeae* and *Neisseria meningitidis* arises from a known nonpathogenic ancestor through phylogenetic theory, and now represent various lineages that normally take over noticeable niches, nasopharyngeal, mucosa, and the genital mucosa relatively (Maiden, 2008; Joseph *et al.*, 2011; Bratcher *et al.*, 2014; Maiden and Harrison, 2016). Although *Neisseria meningitidis* may endure dehydration, it can only survive briefly outside the body of a vulnerable host and is spread through droplet respiration. When *N. gonorrhoeae* is dehydrated to non-physiological temperatures, it can become non-viable. It is still unclear what factors contributed to the emergence of two distinct creatures with roughly related core physiologies and genomes, but separate infections in various areas of the human body. *Neisseria* spp commensal's and pathogenic characteristics share the same microbiota, making it challenging to distinguish between colonization factors and virulence agents that are crucial in causing or contributing to host damage. Since *Neisseria gonorrhoeae* colonizes the rectal, oral mucosa and genital, it activates a repertoire of factors that gives duplication and survival in these environmental niches, and factors that regulate and avoid the host immunity. To facilitate better prevention, diagnostic development, and control of infection as well as the production of vaccines or novel treatments, it is essential to comprehend the phenomena via which *Neisseria gonorrhoeae* interacts.

MATERIALS AND METHODS

***Study area*:** The study was carried out at the Ibrahim Badamasi Babangida University Specialist Hospital and General Hospital Minna, Nigeria. Minna is a city with an estimated population 463,000 in 2021 which has a coordinate of Latitude: 9^0 36’ 29.99’’ N Longitude: 6^0 32’ 51.94’’ E.

***Ethical approval*:** The study was conducted after it was ethically reviewed and approved by the Research Ethical Committee of the Ministry of Health, Niger State Government of Nigeria (NSMOH ERC) with the Approval Number: ERC PIN/2022/03/22.

***Study design*:** This research entails collection of samples from adult male and female patients attending the hospitals mentioned above, with the age range of ≥ 10 years old patients.

***Inclusion criteria*:** The study includes adults with suspected STDs at the microbiology lab for high vaginal swabs (HVS), endo cervical swabs (ECS) and urethral swabs culture and sensitivity.

***Exclusion criteria*:** Adults outside the inclusion criteria were excluded from the study.

***Sample collection*:** The consent of the patients was sought, thereafter a total of one hundred and twenty (120) high vaginal swabs (HVS), endo cervical swabs (ESC) were obtained from female patients and urethral swab from male patients by trained medical personnel with a sterile cotton swab sticks. The samples collected were transported to the Microbiology laboratory of General Hospital, Minna, Nigeria, where the analysis was carried out. A questionnaire was administered to each patient to determine their age, sex, occupation,

educational qualification, sexual activity, area of domicile and antibiotics use as the likely socio-demographic factors that could be associated with the presence of bacteria isolates.

***Isolation of bacteria*:** The samples collected were examined under the microscope for their consistency in colour under x40 high power objective lens and x10 low power objective lens to check the presence of white blood cells, red blood cells and epithelia cells. The samples were then inoculated onto a chocolate agar and a modified chocolate agar plates by streaking method, with the addition of antibiotics such as vancomycin, and nystatin to inhibit the growth of other fastidious bacteria. The plates were incubated under a 5-10% CO_2 enriched atmosphere and humid state for 24-48 hours at 37^0C (Thorley and Radclie, 2015).

***Characterization and identification of the bacterial isolates*:** The bacterial isolates were identified based on their cellular morphology, colonial morphology via Gram stain and subjection to different biochemical tests such as: catalase, oxidase, coagulase, Methyl-red, Voges -proskauer, indole, motility and sugar fermentation tests for further identification (Cheesbrough, 2006)

***Antibiotic susceptibility testing of bacteria isolates*:** The Kirby-Bauer agar disc diffusion technique was used to determine the antibiotic susceptibility of the bacteria isolates (EUCAST, 2021). The plating medium was Muller- Hinton agar, which was prepared according to the manufacturer's instructions. A sterile wire loop was used to pick a small amount of the isolate and spread it on the medium, followed by placing the antibiotics. The antibiotics used included tarivid (OFX 10 µg), ciprofloxacin (CPX 10 µg), streptomycin (S 30 µg), gentamicin (CN 10 µg), Septrin (SXT 30 µg), chloramphenicol (CH 30 µg), erythromycin (E 30 µg), pefloxacin (Pef 5 µg), augmentin (AU 30 µg), ampicillin (AM 20 µg). Zinacef (Z 30 µg), rifampin (RP 5 µg), amoxil (AMX 20 µg) and ampiclox (APX 20 µg). After incubating the plates for 24 hours at 37°C, the zone of inhibition was measured using a meter rule.

Statistical analysis: This was carried out by using chi-square analytical package to ascertain the association between the risk factors and the occurrence of *Neisseria gonorrhoea* and other isolates among patients attending some selected Hospitals in Minna Niger state Nigeria.

RESULTS

A total of one hundred and twenty (120) high vaginal swabs (HVS), endocervical swab (ECS) and urethral swab were collected for the period of 8 weeks from April to June 2022. The samples collected were investigated for the incidence of *Neisseria gonorrhoeae* among patients attending some selected hospitals in Minna, Nigeria. Among the 120 samples collected, 81 (67.50%) were HVS, 16 (13.33%) were ECS and 23 (19.17%) were Urethral swabs.

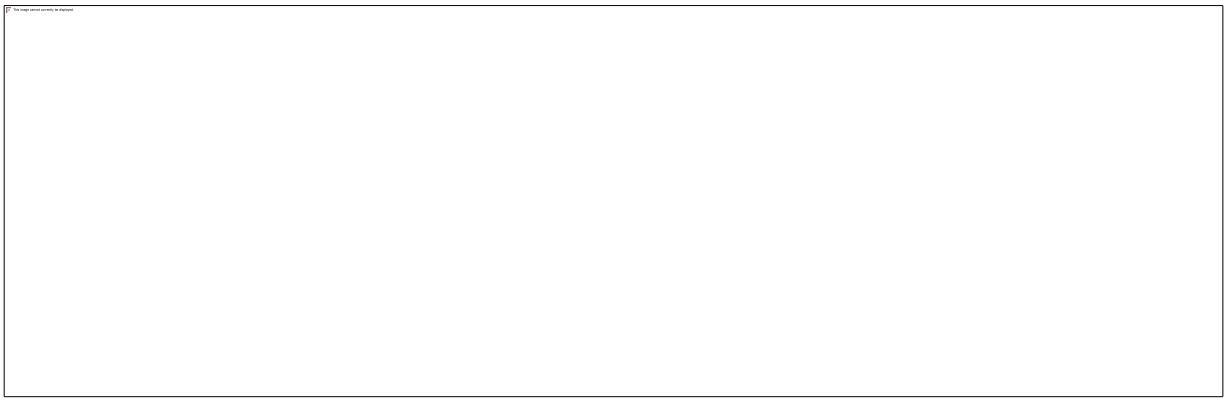

Figure 1: Map indicating local government areas of Niger State, Nigeria.

Incidence of *Neisseria gonorrhea*/other bacterial isolates

The number of samples obtained from General hospital, Minna and IBB specialist Minna, Nigeria gave a total of 120 samples with no positive isolation of *N. gonorrhoeae* from the patients, but the growth of other bacterial isolates from the samples was strictly observed and isolated. Sixty- nine 69(57.5%) samples with 24(62%) positive growth of isolates was collected from General Hospital Minna, 51(42.5%) samples were collected from IBB Specialist Minna, having 15(38%) of positive isolates. A total of thirty-nine (39) isolates was observed at the end of the research, the highest been *E. coli*, 13(33.3%) closely followed by *S. aureus* 12(30.8%), and the least number of isolates was *Klebsiell*a sp. 2 (5.1%) as shown in Table 1 below.

Antibiotic susceptibility pattern of bacterial isolates

The profile of the antibiotic susceptibility of the isolates showed a series of resistance and susceptibility to some antibiotics. The *E. coli* having the highest percentage of occurrence 13(33.3%) was highly susceptible to ciprofloxacin (77%), and resistant to streptomycin (8%). *Pseudomonas aeruginosa* was highly susceptible to ciprofloxacin (100%) and highly resistant to chloramphenicol (89%). *Klebsiella* sp. was highly susceptible to ciprofloxacin, septrin and ofloxacin by 100%. While it was highly resistant to chloramphenicol (100%) (Table 3) The S. *aureus*was moderately susceptible to ciprofloxacin (58%) and highly resistant to zinacef, amoxil, and erythromycin. *Streptococcus* sp.was highly resistant to amoxil, rifampin, erythromycin and gentamicin (100%), but susceptible to septrin and ciprofloxacin (67%) (Table 2).

Sociodemographic factors associated with some bacterial infections among the study participants

Statistical analysis of the socio-demographic factors considered in the research indicated that occupation of the patients attending IBB specialist and General hospital Minna is not statistically a factor at P <0.05 to the occurrence of the bacteria isolates in the patients, having the P value of 0.192527 (P > 0.05). This implies that Occupation is not associated with the occurrence of the bacterial isolates in the patients (Fig 2). Marital status of the patients is a factor to the occurrence of the bacterial isolates in the patients with the P-value of 0.00001 (P<0.05). This means that marital status is associated with the occurrence of bacterial isolates in the patients (Figure 5). Area of Domicile of the patients indicated that the P-value is equal to 0.00001 (P < 0.05).This indicated that there is an association between the occurrence of the bacterial isolates and the area of domicile of the patients (Figure 4).Sexual activityof the patients indicated that the P- value of < 0.05 is equal to 0.00001 which indicates that there is an association with the positive results. The statistical analysis indicated that age of the patients attending the hospitals is associated to the occurrence of bacterial isolates in the patients. The P-value was found to be 0.00082 (P< 0.05) (Figure 1). The statistical analysis also indicated that Educational status has no association with the presence of the bacterial isolates in the patients across the hospitals. The P - value = 0.19252 (P> 0.05) (Figure 3).

Table 1: Occurrence of bacteria in the HVS, ECS and US of the patients

Microorganisms	Number of Isolates	% Occurrence
Escherichia coli	13	33.3
Klebsiella sp.	2	5.1
Pseudomonas aeruginosa	9	23.1
Staphylococcus aureus	12	30.8
Streptococcus sp.	3	7.7
Total	**39**	**100**

Key: HVS= High vagina swab, ECS= Endocervical swab, US= Urethral Swab; Formula = Number for each organism ÷ Total number of all isolated organism × 100

Table 2: Antibiotics susceptibility profile for Gram positive bacteria isolates

Antibiotic/Bacteria	Disc Ct (µg)	*Staphylococcus aureus* n (12) (%)	*Streptococcus* sp. n (3) (%)
Ciprofloxacin	10	58(42)	67(33)
Streptomycin	30	33(67)	33(67)
Co-trimoxazole	20	33(67)	67(33)
Pefloxacin	5	50(50)	33(67)
Erythromycin	30	0(100)	0(100)
Gentamicin	10	42(58)	0(100)
Ampiclox	20	17(83)	33(67)
Zinacef	30	0(100)	33(67)
Amoxil	20	0(100)	0(100)
Rifampicin	5	8(92)	0(100)

Key: Numbers inside the bracket = Percentage Resistance, Number outside the bracket = Percentage Susceptibility, Ct = Content, D = Diameter

Table 3: Antibiotic profile for Gram negative bacteria

Antibiotic/Bacteria	**Disc Ct (µg)**	***Escherichia coli* n= (13) (%)**	***Pseudomonas aeruginosa* n= (9) (%)**	***Klebsiella* sp. n= (2) (%)**
Ciprofloxacin	10	77(23)	100(0)	100(0)
Ampicillin	20	15(85)	33(67)	0(100)
Gentamicin	10	15(85)	44(56)	50(50)
Sparfloxacin	5	15(85)	33(67)	50(50)
Cotrimoxazole	20	23(77)	22(78)	100(0)
Chloramphenicol	30	15(85)	11(89)	0(100)
Streptomycin	30	8(92)	44(56)	50(50)
Pefloxacin	5	23(77)	56(44)	50(50)
Ofloxacin	5	38(62)	0(100)	100(0)
Augmentin	30	8(92)	0(100)	50(50)

Key: Number outside the bracket = Percentage susceptibility, Number inside bracket = Percentage resistance, D = Diameter, Ct = Content

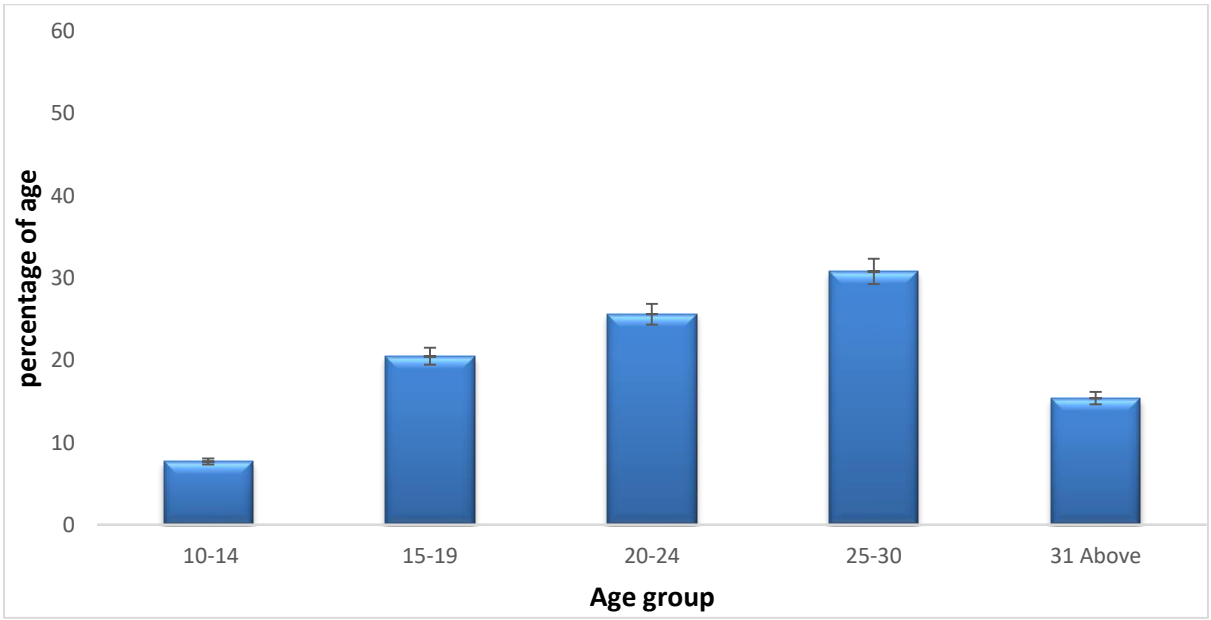

Figure 2: Occurrence of bacterial isolates by age group (P-value = 0.00001, P < 0.05)

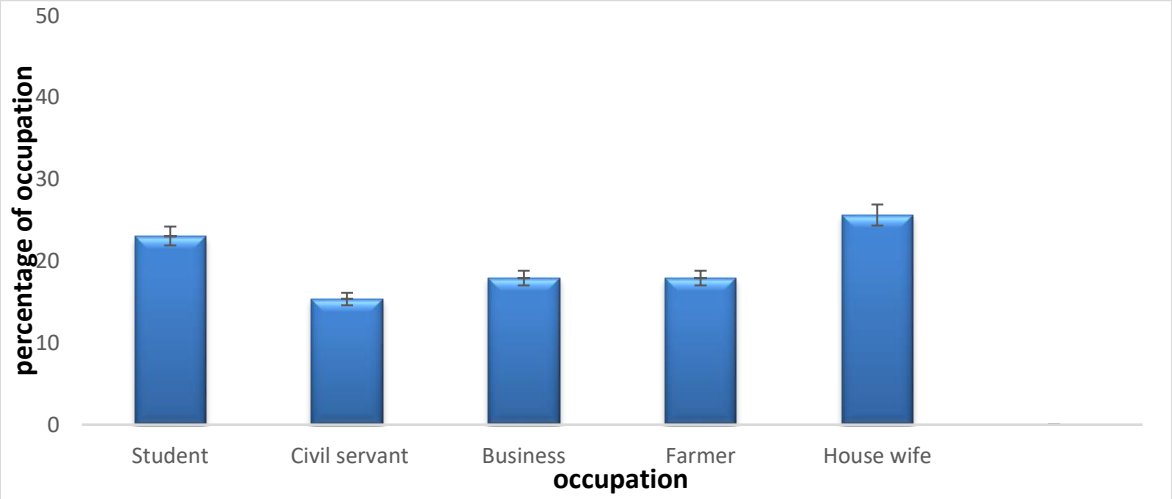

Figure 3: Occurrence of bacterial isolates among patients by occupation (P-value = 0.19252, P < 0.05)

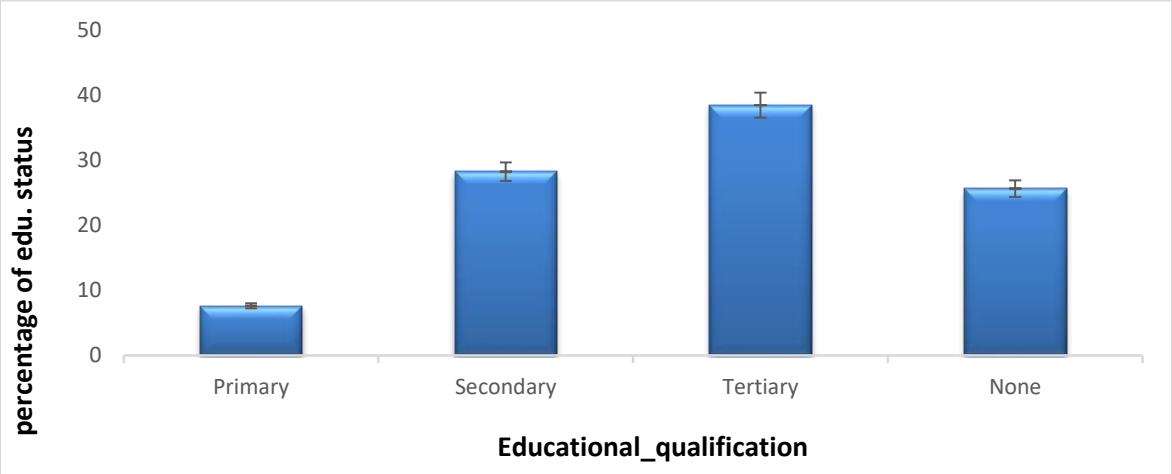

Figure 4: Occurrence of bacterial isolates among patients by educational status (P-value = 0.192527, P > 0.05)

Figure 5: Occurrence of bacterial isolates among patients by area of domicile (P-value = 0.000082, P < 0.05)

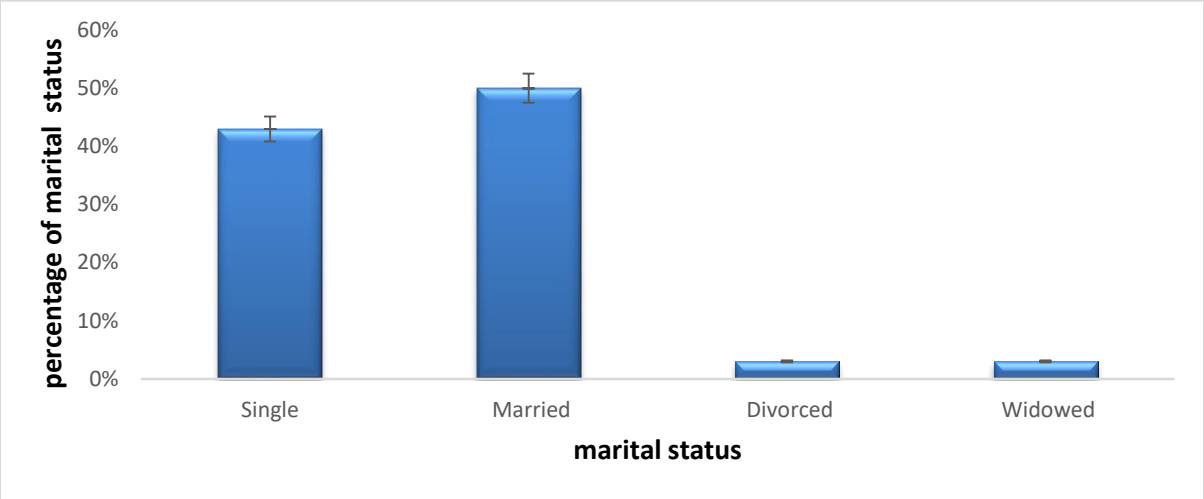

Figure 6: Occurrence of bacterial isolates among patients by marital status (P-value = 0.00001, P < 0.05)

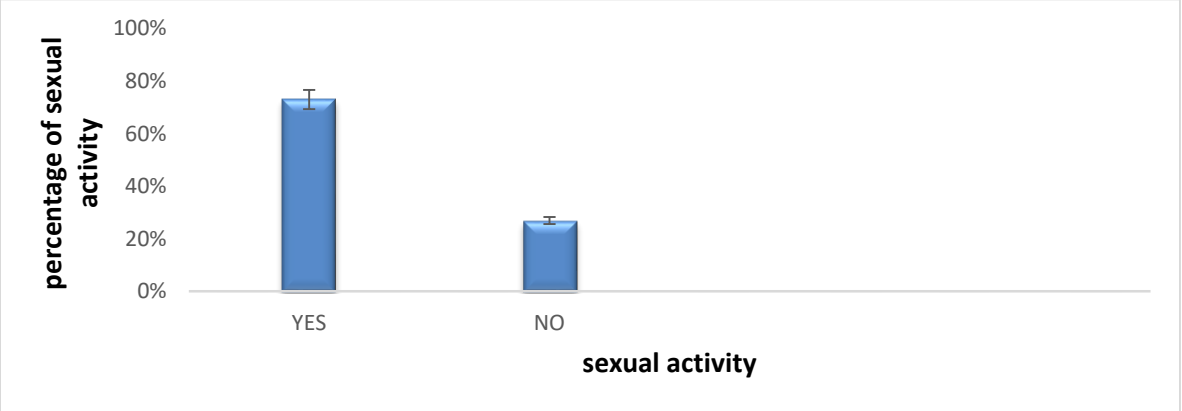

Figure 7: Occurrence of bacterial isolates among Patients' by Sexual active (P-value = 0.00001, P < 0.05)

DISCUSSION

The study investigated the incidence of *Neisseria gonorrhea* infection among patients attending IBBU Specialist and General Hospital Minna, Niger State Nigeria. The non-occurrence of *Neisseria gonorrhea* within the period of April-June 2022 in this study differs from the 1.4% discovery in a recent study conducted in Owerri, Nigeria (Nsofor and Eletuoh, 2017). In another study, only four isolates of *Neisseria gonorrhea* were obtained in two major Hospitals in Minna Nigeria, amounting to 5.56% (Kenneth, 2005), and one isolate (1.3%) in Illorin (Aboyeji and Nwabusi, 2003), and also in Abuja by 3.2% (Bassey *et al.*, 2000). Other isolates from HVS, ECS and urethral swab in this study includes, *Escherichia coli* which predominates, amounting to 13(33.3%) isolates. This agrees with the findings of Odoki *et al.* (2019) having a high prevalence of *E. coli*, 41.9%. In a related study in a Ugandan community of Mulago, a high incidence of *E. coli*, 50% was reported (Mwaka *et al.*, 2011; Kabugo *et al.*, 2016). Similar study by Ejerssa *et al.* (2021) reported that high incidence of *Escherichia coli* was attributed to it being a bowel commensal. This is due to the fact that commensals of the intestine were more involved in UTI due to its anatomic proximity to the genito-urinary area. However, this study contradicts a study from Minna, Niger State, Nigeria where *Klebsiella pneumoniae* was found at the highest frequency (39.1%) (Kenneth, 2005).

In this case, *Klebsiella pneumoniae* was the least observed isolate (5.1%), after *Streptococcus* sp. The observed difference could be attributed to the endemicity of the isolate in the community, as well as the climatic and geographic variation of the study sites (WHO, 2015). The occurrence of *E. coli* in this study may be due to fecal contamination as a result of proximity of the genital areas to the anus; this Organism might be transferred accidentally through sexual contact. This study also reported *S. aureus* as one of the second most prevalent urogenital pathogen with 12 isolates (30.8%), which is comparable to previous studies in Awka, Nigeria (Ekwealor *et al.,* 2016). The occurrence of *Pseudomonas aeruginosa* (23.1%) in this studies agrees with the findings of Kenneth, (2005) that reported *Pseudomonas aeruginosa* as the third leading cause of Hospital acquired UTIs amounting to 12%. It can also be found in the blood stream to the rate of 40% *Pseudomonas* bacteremia.

The antibiotic Susceptibility test was determined based on zone of inhibition in line with CLSI (2016). However, the result of this study indicated that *E. coli* isolated was susceptible to ciprofloxacin and pefloxacin. Other isolates sensitive to this antibiotic includes, *Pseudomonas* sp., this agrees with the work of Hooton *et al.* (2012) where ciprofloxacin was effective against *Pseudomonas aeruginosa*. Recent researches also revealed that the success of ciprofloxacin was due to its broad spectrum activities and its activity to disrupt DNA functions leading to death of the bacterium (Vorland, 2001). This is contrary to previous reports on some Antibiotics where most isolates were shown to be susceptible to Pefloxacin which could be a development to metabolic pathway of the microorganism.

Resistance to antibiotics in this study indicated increase of resistance among bacteria isolated, possibly causing urogenital tract infections (Abbas *et al.,* 2017). S*taphylococcus aureus* isolates showed 100% resistance to Ampicillin and erythromycin. This finding is supported by Alo *et al.* (2015). The improper use or prescription of these drugs should be avoided to reduce or eliminate antibiotics resistance by microorganisms (Elvis *et al.,* 2018). An increase in resistance to antibiotics by microorganisms could also be due to rapidly growing population to progressive urbanization in the community and increase in consumption rate of the drugs (Abbas *et al.,* 2017).

This study indicated that there is an association between the factors (age, marital status, sexual activity, and area of domicile) and the presence of bacterial isolates in the vagina, cervix and urethral of patients ($P < 0.05$). Sexual activity enhances the transmission of urogenital tract infections, which is likely to affect women the more, due to anatomy, making it easy for bacteria to enter every opening in the urogenital tract (Meryl and Kacy, 2020). Marital status is another factor to be considered in line with the incidence of genitourinary tract infection among patients, married people were more prone to this infection. This fact was supported by the findings of Boskey (2022). Area of domicile also favored genitourinary tract infection, the participants who resides in town had greater chances. This may be due to higher involvement in social activities in town dwellers compared to those who stay in villages. The study also indicated that there was no association between the factors (Occupation and education) and the presence of the bacterial isolates from the patients ($P > 0.05$). This finding was supported by the work of Kadhim (2018).

CONCLUSION

From the result of this research, *Neisseria gonorrhea* was not isolated from the samples obtained from the patients. However, *E. coli* and *S. aureus* were the predominant pathogens isolated with the highest number of isolates. This could be interpreted that not all vagina discharge or dysuria is caused by *Neisseria gonorrhea,* but can be due to the presence of other pathogens. It is possible that every patient who come to the hospital with discharge and pelvic pains are being treated with empirical/herbal drugs, while some people with sudden symptoms prefer to treat themselves at home due to privacy and shy to be known with such infection.

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