UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp.154 - 161

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.018

Received: 13th March, 2024 **Accepted:** 17th June, 2024

Prevalence of Multi Drug Resistant Coagulase Negative Staphylococci among some Pregnant Women Attending Antenatal Clinic in Yola, Nigeria

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Abstract

Coagulase negative staphylococci have been reported to be a frequent agent of uncomplicated UTI in young sexually active women which can also lead to symptomatic infection, low birth weight (LBW) babies and preterm delivery. This study was undertaken to determine the prevalence and characterize coagulase negative staphylococci among pregnant women in public health facilities in Yola. A total of 250 urine samples were collected from pregnant women attending antenatal clinic at Specialist Hospital and Boshang Clinic and Maternity both in Yola. The samples were cultured on cysteine lactose electrolyte deficient medium and isolates characterized using phenotypic methods. The prevalence of coagulase negative staphylococci from this study was 26.97%, Staphylococcus aureus 16.29% and Escherichia coli 36.51%. The frequency of CoNS was highest among age group of 36-40 years and least among 16-20 years (12.5%). CONS isolated from this study demonstrated high susceptibility to pefloxacin, ciprofloxacin, erythromycin, ceftriaxone and streptomycin. Some isolates showed high resistance to ampiclox, cefuroxime and amoxicillin. About 83% of the isolates had multiple antibiotic resistant indexes greater than 0.2 with about 25% of the antibiotic resistance being plasmid mediated. Furthermore, phenotypic screening using the double disk synergy test showed that 20 (40%) of the isolates were extended spectrum beta lactamase (ESBL) producing. This study revealed the presence of multi-drug resistant and ESBL producing coagulase negative staphylococci among pregnant women in the study area. There is therefore the need for pregnant women to be screened for coagulase negative staphylococci to avoid complications during gestation period.

Keywords: Coagulase negative, Staphylococcus, Pregnant women, Prevalence, Multidrug resistant

INTRODUCTION

Coagulase-negative staphylococci (CoNS) are gram-positive cocci with a characteristic "grape-like" shape and are distinguished from *Staphylococcus aureus* by their lack of coagulase production. They are part of the normal human flora but can sometimes cause infections, particularly in individuals with implanted devices, the elderly, young children, sexually active women, and immunocompromised patients (Deyno *et al.*, 2018; Ali *et al.*, 2019; França *et al.* 2021). While there are approximately 45 recognized species of CoNS, *Staphylococcus epidermidis*, *Staphylococcus lugdenensis*, *Staphylococcus* haemolyticus, and *Staphylococcus saprophyticus* are commonly associated with clinical diseases (Ali *et al.*, 2019; França *et al.* 2021).

Reports have shown that Coagulase negative staphylococci is second only to *E. coli* as the most common causative organism in uncomplicated urinary tract infection (UTI) in young sexually active women. Ali *et al* (2019) have reported prevalence of CoNS to be 100% from Kano while 62.7% was reported in Ilorin by Kolawole (2012). Nicolle (2019) isolated *S. saprophyticus* from 173 (22 %) of 787 study subjects with bacteriuria . The study also showed that 42.3% of the infection occurring among women aged 25-31 years. *E. coli*, *S. aureus* and *S. saprophyticus* were the commonly encountered isolates from the study. Pregnant women aged 26-30 years had more infection than women aged 20-25 years, this they attributed to high sexual activity within that age bracket.

Coagulase negative staphylococci reportedly colonize the rectum and genitourinary tract in

an age- and season-dependent manner (preferentially in rainy season). Dhukaa (2012) reported that 6.9% of the urogenital tract of healthy women is colonized (median age, 29 years) from an outpatient gynaecology clinic. In 40 % of humans however, the major reservoir of CoNS is the gastrointestinal tract. In sexually active women, Coagulase negative staphylococci are reportedly the common causes of urinary tract infection where they cause asymptomatic infection from the onset and progresses to form symptomatic disease known as cystitis. CoNS are reported to be the etiologic agent for more than 20% of UTI in pregnant women which can lead to symptomatic or chronic infection. Urinary tract infections caused by CoNS and other agents cause mild to severe health challenges on the mother, foetus and neonates with acute pyelonephritis, septicaemia, nephrolithiasis, endocarditis, neonatal sepsis, bacteremia, acute urinary tract infections, urethritis, dysuria, preterm delivery and low birth weight being the severe complications. Increased parity or age, lack of prenatal care, Low socioeconomic status, poor personal hygiene and sickle cell trait are some of the predisposing factors of UTI in pregnant women (Jain *et al.*, 2013; Nicolle, 2019). Other factors that may lead to UTI may include functional urinary tract abnormalities and diabetes mellitus. Because of the concerns associated with coagulase negative staphylococci infections in pregnancy, we conducted this study to determine the prevalence of CoNS among pregnant women in Yola, Nigeria.

MATERIALS AND METHOD

ETHICAL APPROVAL

Approval to conduct this study was sought and obtained from the management of the various hospitals and clinics from where samples were collected. The reference number for the approval was MAU/MB/SHY/07/710/vol.1. Informed consent was obtained from all study subjects, and it served as the basis for their enrollment in the study.

Study Population

The subjects enrolled in this study were pregnant women that registered for ante natal clinic in public or private hospital in Yola irrespective of age, tribe and socioeconomic status that willingly volunteered to be included in the study

Sample Size

A total of 250 randomly collected urine samples from pregnant women showing no signs of urinary tract infection attending antenatal clinics in private and public hospitals in Yola, Nigeria, were randomly selected for this study while ensuring their confidentiality. An average prevalence of 80% reported by Ali *et al* (2019) and Kolawole (2012) was utilized to calculate the sample size using the formula N= Z^2 pq/d^2, where:

N= sample size

Z = Standard normal deviate at a 95% confidence interval.

P = Proportion of the target population

q= 1- p

d= degree of freedom.

Z = 1.962, p = 0.80, q = 1 - 0.80 = 0.20, and d = 0.052.

Thus;

N= 0.6159110/0.0025 =246.4

Therefore, a total sample of 246.4 was required, and this was rounded up to 250.

Sample Collection and Processing

The study subjects were instructed on how to collect the urine specimen following the methods reported by Chessbrough (2006). Briefly, early morning clean catch mid-stream urine samples were collected into sterile universal container containing boric acid. The urine sample was then labelled properly with information of age, occupation, parity, gestational age and be transported to the laboratory and processed using standard methods.

Urine Culture

The urine samples were inoculated on Cysteine Lactose electrolyte-Deficient (CLED) medium using a sterile wire loop. The inoculum was spread thinly over the plate area using the streaking method to allow for discrete colony growth. The plates were then incubated at 37 °C for 24 hours. The morphological characteristics of the bacterial growth were observed, and the colonies were counted to determine significant bacteriuria (Prescott *et al.*, 2008).

Characterization of Bacterial Isolates

After the incubation period, isolates were identified using microscopic and biochemical tests. The biochemical tests conducted included catalase, coagulase, urease, oxidase, trehalose utilization tests, haemolytic test, novobiocin susceptibility, etc., as described by Chessbrough, (2006).

Antibiotic Susceptibility Test

Antibiotic susceptibility testing of pure cultures of confirmed isolates will be conducted on Mueller Hinton agar using the Kirby-Bauer disc diffusion method following the guidelines outlined in CLSI, (2017). To begin, a suspension of the fresh isolate will be prepared in sterile normal saline and standardized to a 0.5 McFarland standard. Subsequently, 0.2 ml of the bacterial suspension will be transferred onto molten Mueller-Hinton agar and evenly spread using a sterile glass spreader. Antibiotic discs obtained from Oxoid (Hampshire, United Kingdom) will then be placed on the agar using sterile forceps. The discs include Pefloxacin (5 µg), Gentamycin (30 µg), Ampiclox (20 µg), Cefuroxime (10 µg), Amoxicillin, Ceftriaxone (10 µg), Ciprofloxacin (5 µg), Streptomycin (10 µg), Cotrimoxazole (25 µg), and Erythromycin (15 µg). Following incubation at 37 ºC for 24 hours, the plates will be examined for the diameter of the zone of inhibition around each antibiotic disc. The zone diameters will be measured in millimeters and compared with a zone diameter interpretation chart provided by CLSI, (2017).

Screening of bacterial isolates for Extended Spectrum Beta Lactamase (ESBL) production

Coagulase-negative *Staphylococcus* isolates were screened for extended-spectrum beta-lactamase production following the guidelines outlined in CLSI, (2017). Individual antibiotic discs containing cefotaxime (30 µg) and ceftazidime (30 µg) were aseptically placed 30 mm apart on MH agar plates inoculated with the test organism and incubated for 18-24 hours at 37°C. After the incubation period, the zones of inhibition were measured and recorded to the nearest millimeter. ESBL production was suspected if any of the test bacteria exhibited a diameter of less than 27mm for cefotaxime and less than 22mm for ceftazidime susceptibility, or if they were resistant to any third-generation cephalosporins (cefotaxime and ceftazidime) (CLSI, 2017). Suspected ESBL-producing isolates were then confirmed for ESBL production using the double-disk synergy test as described by CLSI, (2017).

Biofilm Formation Test

The Congo red screening for biofilm formation was adopted. The medium was prepared with 37 g brain heart infusion broth, 50 g sucrose, 10 g agar, and 0.8 g Congo red per liter. Congo red stain was prepared as a concentrated aqueous solution, autoclaved at 121°C for 15 minutes, and then added to the other components of the culture medium when it cooled to 55°C. Plates were then inoculated with one or more colonies of the original isolate and incubated at 37°C for 24 hours. A positive result was indicated by the presence of black colonies on the surface, while strains that did not produce slime developed red colonies (Freeman *et al.*, 1989).

Plasmid Curing Test

Coagulase Negative *Staphylococcus* spp were screened for the presence of plasmids using the 10% sodium dodecyl sulphate (SDS) method described by Mirmomeni *et al.*, (2007). Isolates of Coagulase Negative *Staphylococcus* spp will be grown for 24 hrs and incubated at 37 °C in nutrient broth containing 10% SDS. After 24 hrs, the broth culture will be agitated to homogenize the content, and a loopful of the broth culture will then be subcultured onto Mueller Hinton Agar (MHA) plates, and antibiotic sensitivity testing will be carried out as previously described. Absence of a zone of inhibition on Mueller Hinton agar will indicate plasmid-mediated resistance (plasmid cured), while the presence of a zone of inhibition on Mueller Hinton agar indicates chromosome-mediated (plasmid not cured). Cured markers will be determined by comparison between the pre- and post-curing antibiogram of the isolates.

RESULTS

Results in Table 1 show that a total of 178 isolates were obtained from this study, with *Staphylococcus saprophyticus* being the only coagulase-negative staphylococci, accounting for 26.8% of the isolates. The other uropathogens isolated from the study include *Escherichia coli* 65(36.51%), Staphylococcus *aureus* 29(16.29%), *Klebsiella pneumonia* 19(10.67%), and *Proteus mirabilis* 17(9.55%).

The distribution of coagulase negative staphylococcus infection in relation to age of study subjects is shown in Table 2. The highest occurrence of coagulase negative

staphylococcus infection is seen in the age group of 36-40 years followed by age group >45 yrs. 25%. and the lowest occurrence was seen in the age group 16-20 yrs. (12.5%).

Table 3 shows the distribution of coagulase negative staphylococcus in relation to gravidae. The highest occurrence of coagulase negative *Staphylococcus* infection was observed in the primigravidae while the multigravida had the least occurrence of coagulase negative staphylococci.

Coagulase-negative staphylococci were observed to be more prevalent among pregnant women in the third trimester (26.7%) but least common in the first trimester of pregnancy. The variation in the occurrence of coagulase-negative staphylococci was not statistically significant at p=0.05 (Table 4).

The results of the antimicrobial susceptibility screening of coagulase-negative staphylococci revealed that the majority of the isolates (over 70%) were susceptible to pefloxacin, ceftriaxone, ciprofloxacin, streptomycin, and erythromycin. Similarly, over 70% of the isolates demonstrated resistance to ampiclox, cefuroxime, and cotrimoxazole (Table 5).

Table 1: Percentage of occurrence and distribution of uropathogens among pregnant women

Isolates	No of isolates	Prevalence (%)
Escherichia coli	65	36.5
Staphylococcus saprophyticus	28	15.5
Staphylococcus epidermidis	12	6.6
Staphylococcus aureus	29	16.3
Klebsiella pneumonia	19	10.7
Proteus mirabilis	17	9.6
Total	178	71.2

Table 2 Distribution of coagulase negative staphylococcus in relation to age of the pregnant women

Age interval	No. tested	% positive	P value
16-20	16	2 (12.5)	0.1824
21-25	60	10 (16.7)	P>0.05
26-30	107	20 (18.7)	
31-35	48	8 (16.7)	
36-40	15	7 (46.7)	
>40-45	4	1 (25)	
Total	250	48	

Table 3: Prevalence of coagulase negative staphylococcus in relation to gravidity

Gravidity	No. examined	% positive	P value
Primigravidae	29	22 (75.9)	
Second Gravidae	53	15 (28.3)	P< 0.05
Multiple Gravidae	168	11 (6.5)	
Total	250	48	

Table 4: Distribution of coagulase negative staphylococci in relation to trimester

Trimester	Number positive	Number examined	P value
First trimester	26(17.3)	150	
Second trimester	14 (2)	70	p>0.05
Third trimester	8 (26.7)	30	
Total	48	250	

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

Table 5: Antibiotics susceptibility profile of Coagulase negative *Staphylococcus* isolates

Antibiotics	Susceptible	Intermediate	Resistant
Pefloxacin	42(87.5)	1(2.1)	5(10.4)
Gentamycin	29(60.4)	8(16.7)	11(22.9)
Ampiclox	7(14.6)	1(2.1)	40(83.3)
Cefuroxime	5(10.4)	5(10.4)	38(79.2)
Amoxicillin	10(20.8)	5(10.4)	33(68.8)
Ceftriaxone	34(70.8)	1(2.1)	13(27.1)
Ciprofloxacin	41(85.4)	-	7(14.6)
Streptomycin	34(70.8)	2(4.2)	12(25)
Cotrimoxazole	8(16.7)	3(6.3)	37(77.1)
Erythromycin	34(70.8)	5(10.4)	9(18.6)

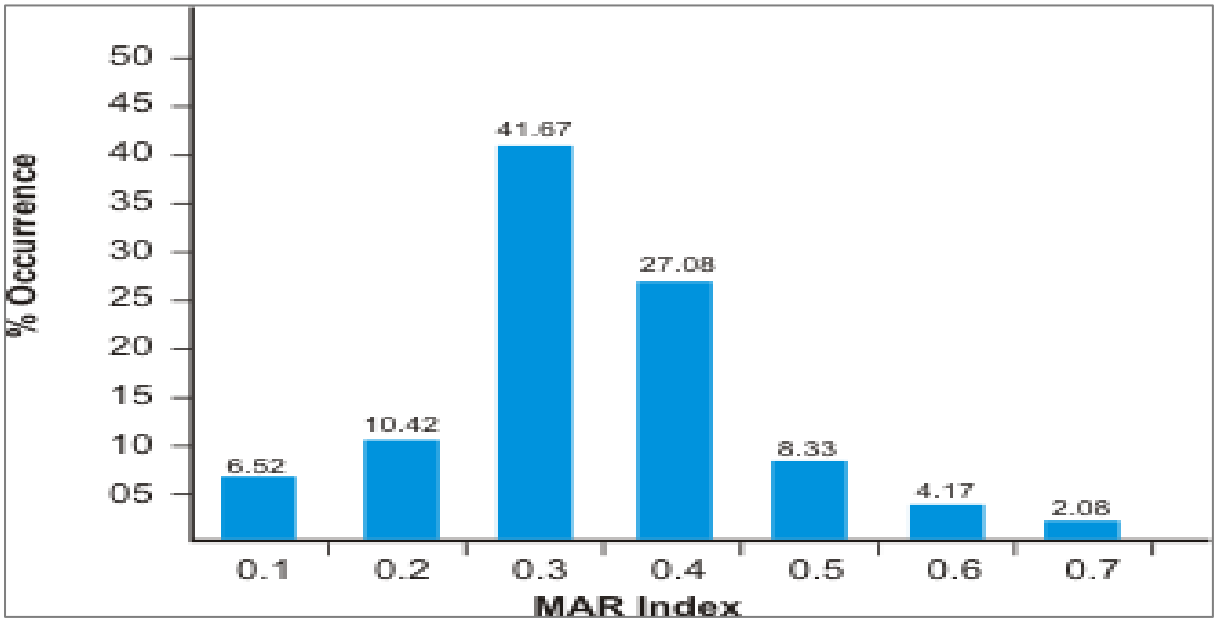

Figure 1 Multiple antibiotics resistance of coagulase negative staphylococci from pregnant women.
Key: MAR- multiple antibiotics resistance

Table 6: ESBL production among bacterial isolates using Double Synergy method

	Presumptive	Confirmed	Negative
S. *saprophyticus*	14	6	8
S. *epidermidis*	6	2	4
Total	20	8	12

Key: Presumptive (Cefpodoxime =≤22mm, Ceftriaxone=≤25mm, and Ceftazidime =≤ 22mm, confirmed using the double disc synergy test by observing the expansion of the indicator cephalosporin inhibition zone towards antibiotics with Clavulanic acid.

DISCUSSION

The prevalence of urinary tract infection due to coagulase-negative staphylococci among pregnant women in this study was 26.8%, second only to *Escherichia coli* with a prevalence of 35.5%. This rate is slightly higher than the 21.6% reported by Matalka *et al*. (2021) but lower than the 42.3% reported by Nicolle, (2019) among women aged 25-31 years. The prevalence of coagulase-negative staphylococci from this study confirms earlier reports of CoNS being the second most common agent of uncomplicated UTI in sexually active women, following E. coli. The physiological changes in a woman's body during pregnancy increase the risk of urinary stasis and vesicoureteral reflux. These changes, coupled with challenges in maintaining proper hygiene due to a distended pregnant belly and a naturally short urethra, contribute to the higher frequency of urinary tract infections during pregnancy.

From the results of this research, the overall prevalence of bacterial isolates was 71.2%. Comparing this result with other studies on the prevalence of pathogens associated with UTI, previous research by Obiogbolu *et al.*, (2009) in Akwa metropolis, South-eastern Nigeria, highlighted *E. coli* as a common urinary tract pathogen, with a reported high incidence of UTIs (54%) in pregnant women. Similarly, Okesola and Oni, (2005) found that the most prevalent uropathogens in urine were *Staphylococcus aureus* (47.5%), *Pseudomonas aeruginosa* (24.6%), *Klebsiella species* (23%), *Proteus spp* (3.3%), and *E. coli (*1.6%). These findings suggest that there has been limited focus on coagulase-negative staphylococci, which studies have shown account for more than 20% of UTIs in pregnant women (Nicolle, 2019).

The highest occurrence of coagulase-negative staphylococcus infection is observed in pregnant women aged 36-40 years (25%). However, this difference is not statistically significant at p=0.05. This could be attributed to the high level of sexual activity within this age group, as sexual activity increases the risk of UTI, and women in this age bracket are typically sexually active. This finding contrasts with the report by Akinloye *et al* (2006) who identified advanced maternal age (≥ 35 years) as a risk factor for asymptomatic bacteriuria in pregnancy.

The highest occurrence of coagulase-negative *Staphylococcus* infection observed in primigravidae can be attributed to the pressure effect of a larger uterus on the ureter and pressure on the bladder from the descending part, leading to stasis of urine and increased multiplication of bacteria. Akinloye *et al* (2006) also reported that multiparity is a risk factor for acquiring asymptomatic bacteriuria in pregnancy. There was a significant difference in the prevalence of asymptomatic bacteriuria with respect to gravidity (P=0.000).

Coagulase-negative staphylococci infection was observed to be more common among pregnant women in the third trimester. This is due to the high level of progesterone produced during pregnancy, which relaxes the urinary tract, thereby causing urine to remain longer in the system, resulting in the growth and accumulation of pathogens. This condition, along with the weight of the growing uterus on the bladder, leads to urinary stasis, thereby increasing the risk of infection during pregnancy. In this study, the peak of infection occurs in the second trimester (54.17%), which agrees with the work of John *et al*, (2018), that shows a higher frequency of CoNS in the second trimester and closely in the third trimester. This study differs from that of Fred *et al*. (2015), who reported a higher frequency of CoNS in the third trimester than in the first and second trimester. Okonko *et al*. (2010) also reported a higher prevalence in the third trimester in a study to detect CoNS among pregnant women in Ibadan, South-Western Nigeria. This difference may be attributed to changes in urinary stasis and vesico-urethral reflux, personal hygiene, or a decrease in urinary hormones in the various trimesters of pregnancy.

Results of the antimicrobial susceptibility screening of coagulase-negative staphylococcus revealed that the majority of the isolates (over 70%) were susceptible to pefloxacin, ceftriaxone, ciprofloxacin, streptomycin, and erythromycin. Similarly, over 70% of the isolates demonstrated resistance to ampiclox, cefuroxime, and cotrimoxazole. The antibiotic susceptibility pattern results show that CoNS were highly susceptible to pefloxacin (87.5%), ciprofloxacin (85.42%), erythromycin, ceftriaxone, and streptomycin (70.83%). This finding is consistent with a previous study by Akinloye *et al* (2006) where *Staphylococcus saprophyticus* showed resistance (100%) to amoxicillin, ampiclox, and ceftazidime, with 66.7% resistance to erythromycin and cotrimoxazole. CoNS have been reported to be resistant to a wide spectrum of antibiotics, with many species commonly resistant to antibiotics

currently used for staphylococcal infections. The use of antibiotics in hospitals has created a reservoir of antibiotic resistance genes and has contributed to the accumulation of resistant coagulase-negative staphylococci strains.

About 80% of CoNS isolates from this study had multiple antibiotic resistance (MAR) indexes above 0.2. This indicates that a significant proportion of the bacterial isolates have been exposed to multiple antibiotics and have consequently developed resistance to these drugs. Moreover, a MAR index exceeding 0.2 suggests that these bacterial strains likely stem from an environment where antibiotics are frequently misused, abused, or of substandard quality.

Presence of 16.7% ESBL-producing isolates among the pregnant women implies the risk of treatment failure even when using third-generation cephalosporin. This will increase the vulnerability of the pregnant women to all risks associated with such organisms on the pregnant women, fetus, and neonates. The presence of plasmids in some of the isolates could also imply possible horizontal transfers to other isolates, thereby further increasing the risk. A few isolates showed resistance after curing, indicating that such resistance was chromosomally borne.

Biofilm formation was observed among the isolates, which is consistent with the known ability of cons to produce slime and adhere to surfaces, particularly indwelling devices. This characteristic makes the clearance of these organisms from the system challenging due to the resistance of the microbial communities to antimicrobial substances.

CONCLUSION

This study has shown a CoNS prevalence of 26.8% in urine samples collected from pregnant women in the study area. The research also confirmed the presence of multidrug-resistant CoNS isolates, with some of the resistance likely being plasmid-mediated. Therefore, it is recommended that pregnant women be screened for CoNS and their antibacterial profiles during their antenatal clinics. Additionally, pregnant women should adhere to good personal hygiene practices to reduce the risk of Staphylococcus infection.

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