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Microbiology Section

Community Acquired Methicillin Resistance *Staphylococcus* Species and Inappropriate Antibiotics use among Women of Reproductive Age Group in Enugu, Nigeria

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ABSTRACT

Introduction: The burden of community acquired antibiotic resistance is increasing with an alarming rate in the developing countries. Many factors, including inappropriate use of antibiotics, have been suggested as the causes of spread of resistant strains in the community.

Aim: To compare antibiotics susceptibility pattern of community acquired gram positive isolates among women of reproductive age with history of recent inappropriate antibiotics use and those with no history of antibiotics use.

Materials and Methods: The present study was a cross-sectional descriptive study carried out from August 2020 to February 2021 at the State Teaching Hospital in Enugu, Nigeria. The participants for the study were 713 apparently healthy women who presented to the family planning unit for preconception care. All the participants were screened for asymptomatic bacteriuria by culturing their Midstream Urine (MSU). The isolated organisms were identified and Antimicrobial Susceptibility (AST) test performed using the Vitek 2. Polymerase Chain Reaction (PCR) was done for the presence of *mecA* gene among methicillin resistant *Staphylococcus* species. The antibiotic susceptibility pattern of the isolates from participants with positive history of recent antibiotic use was compared with the susceptibility pattern of those with no history of antibiotics using Chi-square test.

Results: Out of the 713 participants, 59 (8.3%) had a positive history of recent antibiotics use. Only 1 (1.7%) participant had her antibiotics prescribed by the doctor while 13 (22%) and 45 (76.3%) obtain their antibiotics at the patent medicine dealer shop and through self-medication respectively. The commonest indication for inappropriate use was febrile illness followed by upper respiratory symptoms. Ciprofloxacin was the most abused antibiotics followed by amoxicillin-clavulanic acid. A 20 (83.3%) and 4 (16.7%) had Asymptomatic Bacteriuria (ASB) among those with recent antibiotic use and those with no history of recent antibiotic use respectively. All the Methicillin Resistant *Staphylococcus aureus* (MRSA) was isolated from the participants with recent history of inappropriate antibiotic use. Also, of significant among this group were quinolone resistant gram positive organisms.

Conclusion: The ASB and multidrug resistant gram positive isolates were common among the participants with recent history of inappropriate antibiotic use. There is need to regulate antibiotics use in the community to prevent selection of multidrug resistant organisms.

Keywords: Febrile illness, Multidrug resistant, Quinolone resistant, Significant bacteriuria, *Staphylococcus haemolyticus*, Vancomycin resistant

INTRODUCTION

Staphylococcus aureus and Coagulase Negative *Staphylococcus* Species (CoNS) are among the major causes of bacterial infection in human [1]. The CoNS are increasingly responsible for diverse infections including bloodstream infection in immunocompromised and sometimes in immunocompetent patients [1]. For instance, *S. haemolyticus* which is the second clinical important CoNS after *S. epidermidis* is recently referred to emerging *Staphylococcus* species in some studies [1,2]. *Staphylococcus* spp. are important in both community and hospital acquired infections. In the either of the case, the organisms are prone for developing antimicrobial resistance [2].

Globally, antimicrobial resistance is a big problem [3]. The developed countries are working hard to curb it by enhanced infection control and appropriate use of antibiotics through functional antimicrobial stewardship program [3,4]. In the developing nations, however, such regulatory use of antibiotics is grossly lacking or inadequate and has led to selection of Multidrug Resistance (MDR) strains in the community [4]. Theses strains spread freely because of poor sanitation and inadequate infection control [4].

In Nigeria, studies have shown that policies on antibiotics are rarely implemented [5]. This has resulted to hawking of antibiotics in many communities, purchase antibiotics without prescription and prescription of antibiotics without absolute indication [5,6]. In such condition, targeted therapy is not often the case in many health institutions. There is extensive use broad spectrum and reserved antibiotics such as quinolones without any aetiological diagnosis and AST testing [7]. Studies have shown that the commonest symptom prompting the misuse of antibiotics is febrile illness. Majority of such symptoms are caused by viruses and malaria [7,8].

Asymptomatic bacteriuria is common in women. It occurs in about 4% of adult women [9]. In pregnant women, the prevalence is almost double and there is over 40% progression to overt Urinary Tract Infection (UTI) and complications [9]. The propensity of progression in non pregnant premenopausal women is low. Therefore, the treatment of ASB is only recommended in pregnancy [9]. In periconceptional evaluation, screening for ASB and treatment could go a long way to prevent complicated UTI [9].

This study was designed to compare the AST profile of community acquired gram positive organisms among participants with history of recent inappropriate antibiotic use and those that did not use

antibiotics. It will also assess the prevalent of ASB in the study population. This is not often considered during preconceptional screening. The organisms were isolated from the urine of asymptomatic women of reproductive age who visited family planning unit for periconceptional care. Therefore, the aim of the study was to compare the prevalence of community acquired bacteriuria among patient with history of inappropriate use of antibiotics and those without history of recent antibiotic use. To compare AST profile in the two groups. To assess for the presence of *mecA* gene in the isolated methicillin resistant *Staphylococcus* species.

MATERIALS AND METHODS

The present study was a descriptive cross-sectional study designed to assess the prevalence and the determining factors of community acquired drug resistant staphylococcal and enterococcal ASB. It was carried out from August 2020 to February 2021. The targeted population was apparently well females who presented to the family planning clinic of Enugu State University Teaching Hospital, Nigeria, to access periconceptional services. Some were on one form of contraceptives for child spacing. The hospital is a major tertiary health institution in the state. It serves the state and the neighbouring southeast states on referral basis. Ethical clearance was obtained from the Ethical Committee of Enugu State Teaching Hospital (ESUTHP/C-MAC/RA/034/vol 2/77). The purpose and objective of the study were explained to the respondents. Then, verbal consent was taken from each participant after clearly explaining the purpose of the study.

Sample size calculation: A minimum sample size of 435 participants was estimated using modified Cochran formula for sample size calculation; with a 95% confidence interval, a margin of error of 4.5% and a 29.5% prevalence of ASB in Enugu as reported by Izuchukwu KE et al., [10]:

The Cochran formula is $n_o=\frac{Z^2pq}{e^2}$ where, 'e' is the desired level of precision, 'p' is the (estimated) proportion of the population which has the attribute in the study, 'q' is 1-p. The z-value is found in a Z table. A 10% attrition was added. However, final sample included was 713 participants in the study.

Inclusion criteria: Family planning clients who were within the reproductive age range. Participants who were on preconceptional visit. Family planning client who present to the clinic to discontinue contraception for the purpose of childbearing were included in this study.

Exclusion criteria: Family planning clients who declined consent for the study after due explanation. Family planning clients with positive history of co-morbidity. Family planning clients who were on immunosuppressive drugs. The clients with significant healthcare contact (admission, works in the healthcare, cared for patents admitted in the healthcare) in the last one year were excluded in this study.

Study Procedure

The participants were selected using a simple random sampling method; the research assistant wrote 'YES' and 'NO' on separate pieces of paper. The papers were folded, and the clients were asked to choose any from a small basket. Those that picked the paper with 'YES' on it were selected for the study. This was done on every clinic day for the period of study.

Prior to sample collection, the participants were educated on the procedure for MSU collection using sterile labelled urine container. The content of the education were the initial wiping of the vulva from the front to the back using a sterile wet gauze, parting of the labia to micturate the initial stream of urine into the toilet system and collecting the mid stream into the provided wide lid sterile urine container before completing the micturition of the remaining urine into the water system. Approximately 10 mL of urine was collected from each participant. The samples were properly labelled. A proforma was also administered to obtain the following information: age, level of education, occupation, type of contraceptives, history of recent (at most two weeks prior to the sampling), antibiotics use, indication for antibiotic use and 'who prescribed antibiotics'.

Sample processing: The samples were processed immediately. Each specimen was vortexed for three seconds before culturing on MacConkey agar (fluke, Switzerland) and Blood Agar (BA) (Antec, United Kingdom) using a standard wireloop [1]. On the BA, a loop full of the sample was spread uniformly on the agar plate to enable colony count for significant bacteria estimation. The plates were incubated aerobically at 37°C for 24-48 hours. Upon incubation, significant bactiuria was estimated as previously published by Koneman textbook in Procop GW et al., [1]. The cultures with $\geq 10^5$ colony forming unit (cfu)/mL and above were considered significant and were processed further. While cultures with $<10^5$ CFU/mL were regarded as contaminants and considered non significant [1].

Microscopic examination of the urine sediments, after centrifugation at 1000 rpm for five minutes, was done to assess for pus cells, epithelial cells, red blood cells, cast, etc. The presence of nitrate, pH, and leucocyte esterase were also assesses using Comb-11 (Labnet, USA).

Bacteria identification and antimicrobial sensitivity: The colonies of all isolates were characterised and gram stained. Further bacterial identification and AST were performed using the Vitek 2 system (bioMérieux, France). Antibiotic susceptibilities were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [11]. For the purpose of this study, however, the gram positive isolates were further characterised and presented.

Molecular identification of MRSA: Cefoxitin resistant *Staphylococcus* species (phenotypic methicillin resistant *Staphylococcus* species) isolates were investigated for *mecA* gene as previously described [12]. Deoxyribonucleic Acid (DNA) extraction was done using Zymo Research (ZR) fungal/bacterial DNA miniprep according to the manufacturer's instructions. The PCR mix was made up of 12.5 µL of Taq 2X master mix from New England Biolabs (M0270); 1 µL each of 10 µM forward and reverse primer; 2 µL of DNA template and 8.5 µL nuclease free water. The primer sequence: mec A F: CTGCTATCCACCCTCAAACAG, mec A R: TCTTCGTTACTCATGCCATACA. The cycling condition was as follow; Initial denaturation at 94°C for five minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds and elongation at 72°C for 45 seconds. Followed by a final elongation step at 72°C for seven minutes.

STATISTICAL ANALYSIS

The findings were analysed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) Statistic software for windows (SPSS statistical software V.21 (IBM Corp. 2019). Descriptive statistics were used to analyse socio-demographic details of all participants and presented in a table. Chi-square was used to compare the association between the history of recent antibiotics use and no history of antibiotic use with the independent variables. The p-value <0.05 were regarded as significant.

RESULTS

Most of the clients were in their reproductive age group as shown in [Table/Fig-1]. Their mean age was 34.65±6.48. Almost half of the participants had tertiary education while 6 (0.8%) had no formal education. Also, nearly half were traders. Civil servants contributed to 28.3% of the clients. Over 96.4% of the participants were married and 541 (75.9%) clients visited the unit for the purpose of child spacing. Implant (Implanon and Jadelle) was the common type of contraceptive used by the clients 386 (54.1%).

Characteristics	N=713	Percentage (%)
Age (years)		
<20	-	-
20-29	151	21.2
30-39	401	56.2
40-49	143	20.1
≥50	18	2.5
Level of education		
No formal education	6	0.8
Primary	55	7.7
Secondary	245	34.4
Tertiary	341	47.8
Postgraduate	66	9.3
Occupation		
Housewife	76	10.7
Civil servant	202	28.3
Trader	333	46.7
Farmer	102	14.3
Marital status		
Married	687	96.4
Single	3	0.4
Divorced	4	0.6
Not specified	19	2.6
Purpose of contraceptives		
Child spacing	541	75.9
Emergency	3	0.4
New client	169	23.7
Types of contraception		
Implant (Jadelle)	100	14.0
Injectable	45	6.3
Implant (Implanon)	286	40.1
IUD	103	14.5
Non	179	25.1

[Table/Fig-1]: Characteristics of study participants.

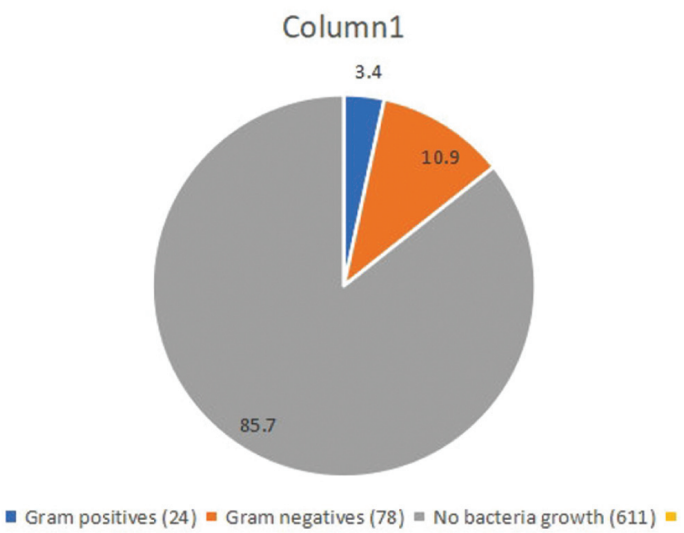
The [Table/Fig-2] represented the history of recent antibiotics use. Out of 59 (8.3%) clients who used antibiotics, only 1 (1.7%) obtained the drug from the hospital, 45 (76.3%) and 13 (22%) used the antibiotics inappropriately by self-medication and without prescriptions respectively. Over 86.4% of the antibiotic use was not supported by laboratory diagnosis or antimicrobial sensitivity assessment. The commonest indication for antibiotic use was a febrile illness, 43 (72.9%) followed by sore throat, 15 (25.4%). Ciprofloxacin was the commonly used antibiotic, contributing to over half of all the antibiotics used by the participants. This was followed by amoxicillin and clavulanic acid.

The [Table/Fig-3] represents the gram reactions of all the isolates. The antibiotic sensitivity profiles of the isolated organisms were shown in [Table/Fig-4]. Two out of nine *Staphylococcus aureus* were cefoxitin sensitive {Methicillin Sensitive *Staphylococcus aureus* (MSSA)}. Two *Staphylococcus aureus* each were Vancomycin Sensitive (VSSA) and Vancomycin Intermediate (VISA) respectively. Most of the organisms were sensitive to nitrofurantoin, linezolid, quinupristin/dalfopristin, and tigecycline. Almost all the isolates were resistant to quinolone. A total of 11 methicillin resistant *Staphylococcus* species were isolated and all of them expressed the *mecA* gene as shown in [Table/Fig-5].

The [Table/Fig-6] showed the association between inappropriate use of antibiotics and age, level of education and occupation of the client, and found no significant difference. The table also represented the relationship of uropathogens isolation and inappropriate antibiotic use. Uropathogens are more likely to be isolated from clients with a recent history of antibiotics use than those with no such history. There was a significant association (p-value=0.001) between inappropriate use of antibiotics and isolating MRSA.

Antibiotic use in the last 14 days	Number	Percentage (%)
Yes	59	8.3
No	644	90.3
I don't know	10	1.4
Name of antibiotics used		
Ceftriaxone	6	8.3
Amoxicillin and clavulanic acid	12	16.7
Ciprofloxacin	41	57.5
Gentamicin	2	2.8
Unknown	11	15.3
Indication for antibiotic use		
Febrile illness	43	72.9
Sore throat	15	25.4
Lower abdominal pain	1	1.7
Antibiotic prescription		
In the hospital	1	1.7
Purchased from patent medicine dealer	13	22.0
Self-medication	45	76.3
Laboratory investigation		
Serology test for typhoid	8	13.6
None	51	86.4

[Table/Fig-2]: History of recent antibiotic usage.
Unknown: as some participants used more than one antibiotic



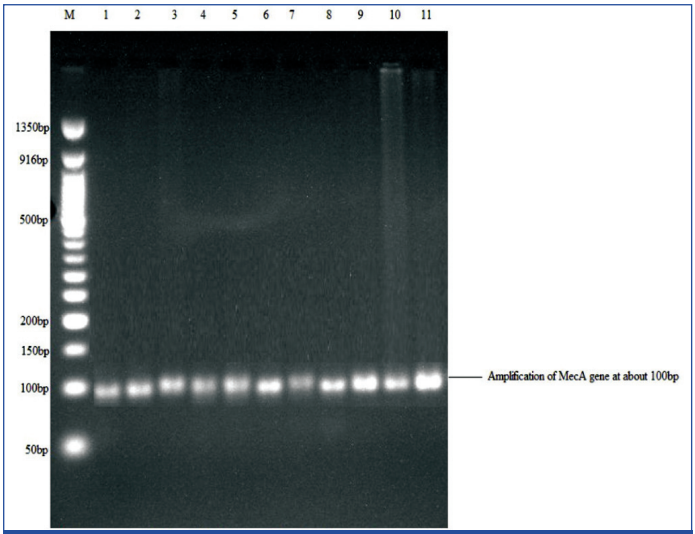
[Table/Fig-3]: The gram reaction of the isolates.

DISCUSSION

The burden of community acquired antibiotic resistance is increasing in developing countries because of the inappropriate use of antibiotics especially in the treatment of non bacterial infectious conditions such as upper respiratory tract infection, viral and parasitic febrile illnesses and viral diarrhoeal diseases [13,14]. The choice of family planning clients as the study population was informed by their negligible contact with the healthcare environment as previously documented [15]. The unit is often situated at the outpatient unit and sometimes outside the healthcare facilities where the clients have no contact with the patients [15]. Also, the majority of the women who visit this facility were in their reproductive age group. In Nigeria, the commonest indication for contraception is child spacing [4]. This means that many of the clients could seek for reversal of contraception to enable them

Isolated organisms	Penicillin	Cefoxitin	Ciprofloxacin/levofloxacillin/ moxifloxacin	Gentamicin	Nitrofurantoin	Clindamycin	Erythromycin	Tetracycline	Tigecycline	Rifampicin	Vancomycin (sensitive)	Vancomycin (intermediate)	Linezolid	Quinopristin/dalfopristin	Ampicillin	Co-trimoxazole
Staphylococcus aureus (n=9)	0	2 (22.2)	1 (11.1)	2 (22.2)	7 (77.8)	2 (22.2)	1 (11.1)	2 (22.2)	9 (100)	7 (77.8)	2 (22.2)	2 (22.2)	7 (7.8)	9 (100)	*	1 (11.1)
Staphylococcus epidermidis (n=1)	0	0	0	0	1 (100)	0	0	0	1 (100)	0	1 (100)	*	1 (100)	1 (100)	*	0
Staphylococcus haemolyticus (n=5)	0	2 (40)	1 (20)	1 (20)	5 (100)	1 (20)	1 (20)	0	5 (100)	1 (20)	5 (100)	*	5 (100)	5 (100)	*	0
Staphylococcus hominis (n=1)	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	*	1 (100)	1 (100)	*	1 (100)
Staphylococcus werneri (n=1)	0	0	0	0	1 (100)	1 (100)	1 (100)	0	1 (100)	1 (100)	1 (100)	*	1 (100)	1 (100)	*	1 (100)
Staphylococcus saprophyticus (n=4)	3 (75)	4 (100)	2 (50)	2 (50)	4 (100)	2 (50)	2 (50)	2 (50)	4 (100)	4 (100)	4 (100)	*	4 (100)	4 (100)	*	3 (75)
Enterococcus faecalis (n=2)	*	*	0	0	2 (100)	*	*	*	*	*	0	*	2 (100)	*	0	*
Enterococcus faecium (n=1)	*	*	1 (100)	1 (100)	1 (100)	*	*	*	*	*	1 (100)	*	1 (100)	*	0	*

[Table/Fig-4]: Antimicrobial Susceptibility (AST) profile of isolated organisms.
*=not determined



[Table/Fig-5]: *MecA* gene amplification.
Gel image showing amplification of *mecA* gene at about 100 bp. The amplicons were resolved on 1.5% agarose gel electrophoresis.
M is a 50 bp ladder

Variables	History of antibiotic uses	No history of antibiotic use	Total	p-value
Age (years)				
<30	23	297	320	0.712*
>30	36	357	393	
Education level				
Secondary school and below	19	287	306	0.09*
Tertiary and above	40	367	407	0.867*
Occupation				
Civil servants	12	190	202	0.333*
Non civil servants	47	464	511	
Staphylococcus aureus				
MRSA	7 (100)	0	7	0.001**
MSSA	1	1	2	
CONS				
MR-CONS	2	2	4	***
MS-CONS	7	1	8	***
***Enterococcus* spp**				
VRE	2	0	2	***
VSE	1	0	1	***
Quinolone testing				
Sensitive	3	1	20	0.001**
Resistant	17	3	4	

[Table/Fig-6]: Inappropriate use of antibiotics with respect to age, level of education occupation and antibiotics resistance.
MRSA: methicillin resistant *Staphylococcus aureus*, MSSA: methicillin sensitive *Staphylococcus aureus*, CONS: Coagulase negative *Staphylococcus* spp, MR-CONS: methicillin resistant coagulase negative *Staphylococcus* spp, MS-CONS: methicillin sensitive coagulase negative *Staphylococcus* spp. p-using Chi-square. *non-significant difference, **significant difference, ***not determined

to get pregnant. In present study, the participants were on their preconception visits [16].

This study observed that 24 (3.4%) of the participants had significant bacteriuria due to gram positive organisms with *Staphylococcus aureus* predominating. This finding obtained was similar to studies among pregnant women [17,18]. Studies have shown that ASB in pregnant women has over 50% chance of progressing to UTI but in non pregnant adults, the probability reduces to 30% [16-18]. The complication and the burden in terms of morbidity and mortality to both mother and child also increase with pregnancy. This is due to both hormonal and structural changes in the urinary tract due to pregnancy [19]. No available study has shown the outcome of ABU in periconceptional women when they eventually become pregnant. Many physicians however screen and treat ABU in women expecting conception to prevent the detrimental complication associated with UTI in early pregnancy [19,20].

Many studies in the country have reported increasing inappropriate use of antimicrobial, with a higher proportion occurring in the community where antibiotics are hawked without regulations [13,14,21-23]. As seen in present study, only one participant had her antibiotic prescribed in the hospital. This is against the World Health Organisation (WHO) recommendation and global action on the implementation of an antimicrobial stewardship program [24]. Many have argued that such programs can only yield the desired result in developing countries when community participation is entrenched [5,24]. From most studies, the commonest indication for irrational use of antibiotics is febrile illnesses and upper respiratory tract

infection [5,24]. Febrile illness in Nigeria is usually viral or parasitic in aetiology, and they do not require antibiotics. Antibiotics stewardship on the other hand requires that the indication for antimicrobial most be defined and evidenced based. It should be supported by the appropriate laboratory investigation [5,24]. This was not the case in this study where only one participant had a doctors' prescription, and few participants had a laboratory test before commencing antibiotics. Although, the laboratory test is no longer recommended for the diagnosis of enteric fever [25].

Nigeria is one of the countries known for the misuse of fluoroquinolones [26]. This involves unregulated access and availability of the drug and the use of substandard and spurious quality of oral ciprofloxacin formulations. It is thought that such use contributed toward increased risk of treatment failure and bacterial resistance in developing countries [26]. The reason for the inappropriate use is high bioavailability, oral formulation, and ease to administer, and its broad-spectrum activity [26]. In present study, ciprofloxacin was the commonly abused drug. Some studies highlighted that it is the second most misused drug in the country following closely the beta-lactams (ampicillin and amoxicillin) [27,28]. The beta-lactams have become the second with an amoxicillin and clavulanic acid, which has a broader spectrum of activity, preferred than ampicillin. This supports the observation by WHO that the world is exhausting its reserve of antibiotics [7].

These frequently irrationally used antibiotics are broad-spectrum, with the unfortunate ability to induce selective adaptation to MDR strains and promote also promote their dominance and spread in communities. This is essential in areas with poor infection control [28]. Ciprofloxacin and other fluoroquinolones are among the restricted antibiotics but its unregulated use could be responsible for its increasing resistance among the gram positive, gram negatives, *Mycobacterium tuberculosis*, etc., [28]. In this study, MDR gram positive organisms were higher in the urine of the participants with a recent history of inappropriate use of antibiotics. Methicillin and quinolone resistant *Staphylococcus* were frequently isolated among this group. Also, isolate were vancomycin resistant *Enterococcus* species such findings have been widely reported in the hospital setting but rarely in the community. Their burden is enormous including increased hospital stay, cost, failure of treatment and increased side-effect [29]. Clinicians usually consider the burden of resistant organisms in their choice of empirical treatment in healthcare associated infections, but such considerations are often not made on community acquired infections [29,30].

Limitation(s)

As in all cross-sectional studies, other factors that contribute to antimicrobial resistant in the community many not have been assessed. Present study also did not assess the effect of the various contraception on the antimicrobial profile of the clients. Also, follow-up the women to know if the isolated organisms were transient colonisers of their UTI. However, the finding of this study will be vital in further investigation in the area.

CONCLUSION(S)

Majority of the participants with the history of antibiotic use obtained the antibiotics without doctor's prescription. Quinolone was frequently used antibiotics followed by amoxicillin-clavulanic acid. The commonest indication for antibiotic use was febrile illness. Methicillin resistant and quinolone resistant *Staphylococcus* and *Enterococcus* spp. bacteriuria were common among the participants with positive history of antibiotics use. There is need to regulate the use of antibiotics in the community through awareness and policy implementation.

Author's contributions: CN supported the study design, led the analysis and drafted the paper; ECA participated in the data collection and helped with analysis; STC participated in the data collection and helped with analysis; PCN participated in data collection, analysis; NEM assisted in analysis and reviewed the draft paper; All authors read and approved the final manuscript.

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