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Prevalence of Methicillin Resistant *Staphylococcus aureus* Bacteriuria among Pregnant Women Attending Secondary Health Hospitals in Ilorin, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary tract infection is one of the most frequently acquired infections in both community and hospitals and is common among the adolescents and the old genders.
Aim: To determine the prevalence of methicillin resistant *Staphylococcus aureus* bacteriuria among pregnant women attending secondary health hospitals in Ilorin, Nigeria
Study Design: An experimental study which involve a random selection of consented pregnant women.
Place and Duration of Study: Department of Biosciences and Biotechnology Kwara State University Malete between January 2018 and June 2019.
Methodology: In this study, a total of 856 pregnant women mid stream clean catch early morning voided urine samples for two consecutive days (383 of the samples were collected from Sobi Specialist Hospital, 278 from Adewole Cottage Hospital and 195 collected from Ajikobi Cottage

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Hospital) were randomly screened for the presence of *Staphylococcus aureus* bacteriuria using standard microbiological procedures such as growth on mannitol salt agar, Gram reaction, catalase and coagulase tests. The Kirby–Bauer disk diffusion method was used to determine the antibiotic sensitivity profile of *S. aureus* isolated using oxoid antibiotic discs.

Results: Out of 856 samples screened 56 samples (6.5%) showed significant *Staphylococcus aureus* bacteriuria, 16- 25 years has a prevalence rate of 5.6%, 26- 35 years (5.8%) while 35-45 years have the prevalence rate of 10.9%. A total of 7 (12.5%) methicillin resistant *Staphylococcus aureus* were isolated. Antibiotic sensitivity profile shows that 26.8% were resistance to gentamicin, 44.6% to tetracycline, 19.6% to chloramphenicol, 33.9 % to erythromycin, 67.9% to amoxicillin, 32% to augmentin, 12.6% to ceftriaxone, 5.3% to ciprofloxacin and 100% susceptibility to both nitrofurantoin and vancomycin.

Conclusion: The study shows the high prevalence of MRSA and high susceptibility of nitrofurantoin and vancomycin to all the MRSA isolated.

Keywords: MRSA; bacteriuria; pregnant women; antibiotics susceptibility, S. aureus.

1. INTRODUCTION

Urinary tract infection (UTI) is among the most frequently encountered acquired infections in both hospitals and communities. This infection is responsible for large global consumption of antibiotics [1]. UTI is caused by microbes that have overcome host defence mechanisms in the urinary tract system which include kidney, ureters and urethra. It is responsible for more than 8 million visitations to clinic and hospitals annually [2,3].

Females are more prone to UTI compared to males due to presence of shorter urethra, increased sexual activity, usage of certain contraceptive methods, proximity of vagina to the anus and non-emptiness of the bladder after urination [4,5].

The major risk factors for recurrent urinary tract infection in premenopausal sexually-active women are the onset of symptoms immediately after sexual intercourse, voiding dysfunction, having many sexual partners, spermicides usage for contraception, young age at the first UTI and maternal history of UTI [6].

UTI is more common in pregnant women compared to non-pregnant women and especially in sickle cell trait carrier and in diabetes mellitus patients [4]. Other risk factors that could increase the prevalence in pregnant women include reduced immune status, urinary stasis, low socio-economic status, grandmultiparity, vesicoureteric reflux presence, old age; hormone induced ureteral dilatation and glycosuria [7,8]. Pyelonephritis can develop as a result of maternal complications which could result in non-essential hypertension, endometritis, preeclampsia, renal scaring and subsequently renal failure [9].

Significant bacteriuria occurs when there is presence more than 100 organisms/mL of urine with associated pyuria in a symptomatic patients or 100,000 organisms/mL of urine in an asymptomatic patient [10].

Staphylococcus aureus, a Gram positive cocci,, catalase and coagulase positive bacteria, has been shown to be one of the main pathogens responsible for urinary tract infections in pregnant women in African countries, together with other microorganisms such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella* and *Staphylococcus saprophyticus.* The microorganisms that cause urinary tract infection, including *Staphylococcus aureus* are responsible for the production of chorioamnionitis and neonatal sepsis in pregnancy [11,12].

It is a pathogen that possesses many virulent factors such as coagulase and toxins which make it responsible for wide range of infections like pneumonia, rashes, endovascular infections, bones and meninges inflammations bones, skin soft tissue infection (SSTIs), septic arthritis, endocarditis, sepsis, osteomyelitis, endocarditis, osteomyelitistoxic shock syndrome and bacteremia [13].

Bacteriuria is one of the major predisposing factor for bacteremia (8 to 34% *Staphylococcus aureus* bacteremia are frequently accompanied by bacteriuria) thus increasing the presence of complications of bacteremia leading to increased risk of intensive care unit admission and mortality [14]. Asymptomatic bacteriuria in a pregnant could result in preterm rupture of membranes

and preterm labour and pregnancy state increases the risk of urinary tract infection and due to many physiological changes. The ureters begin to dilate at around six week of pregnancy (hydronephrosis of pregnancy) which usually peaks at 22-26 weeks and could continue to persist until delivery [15,8]. Estrogens and progesterone levels increase during pregnancy which usually leads to decreased bladder volume and urine concentration which can subsequently result in uretero-vesical reflux and urinary stasis [16].

This study seeks to investigate the prevalence of methicillin resistant *Staphylococcus aureus* bacteriuria in selected secondary health centers in Ilorin, Nigeria.

2. MATERIALS AND METHODS

This is a cross-sectional and descriptive study among pregnant women attending antenatal clinics of three main secondary health centres, these were Adewole Cottage Hospitals, Ajikobi Cottage Hospitals and Sobi Specalist Hospital all in Ilorin, Kwara State between January 2018-June 2019. Ethical approval was obtained from Kwara State Ministry of Health and KWASU Center for Community Development.

Only consented asymptomatic pregnant women were included in the study and all the women were trained on the standard procedure of collecting clean catch midstream urine and supervised by trained Nurse. Exclusion criteria include all pregnant women taking antibiotics and all those with previous complain of urinary tract infections. The collected urine samples in the sterile universal bottles were transported to the Kwara State University, Microbiology laboratory within an hour collection for processing.

MultistixR 10 SG strips (Simens Health Care Private Limited's) were used to analyze midstream urine collected for two consecutive days utilizing the reagent strip method, which detected ten parameters. The process was carried out and the findings were interpreted in accordance with the manufacturer's instructions. The strips came with reagent pads for semi-quantitative testing. Protein, blood, pH, specific gravity, bilirubin, urobilinogen, leukocyte esterase, nitrate, glucose, and ketone were all assessed semi-quantitatively on the strips. Gram's staining was done by smearing a drop of uncentrifuged well mixed urine on a clean grease-free slide, staining it using Gram's technique, and examining it under an oil immersion objective (20 fields). Also urine sample was aseptically inoculated on nutrient agar, MacConkey agar, and mannitol salt agar plates using a conventional technique. When the growth of *S. aureus* in a urine sample is equivalent to or greater than 10^5 colony form unit, significant *Staphylococcus aureus* bacteriuria is determined [17,6]. A considerable bacteriuria of 10^5 CFU/mL of urine is associated with the presence of 1 bacteria per oil immersion field. The isolated *S. aureus* pure cultures were then subjected to species identification and confirmation. Standard laboratory procedures such as Gram staining, colony morphology on mannitol salt agar, catalase, tube and slide coagulase tests, and DNase test were used to confirm the biochemical, morphological, and physiological properties of isolated *Staphylococcus aureus.*

2.1 Antibiotic Sensitivity Test

The Kirby–Bauer disk diffusion method was used to determine the antibiotic sensitivity profile of *S. aureus* isolated using oxoid antibiotic discs. Each of the *S. aureus* isolates was inoculated separately into nutrient broth (Oxoid, UK) and before it was incubated at 37°C for 24 hours before they were tested. Turbidity of the culture was adjusted to correspond with that of a barium sulphate (0.5 McFarland) standard. About 0.1 ml of the nutrient broth culture was later inoculated onto Mueller Hinton agar plates after which it spread over the surface with sterile cotton swabs. Six disks were placed 60 degrees apart on the planted lawn. After 24 hours of incubation at 37°C, the diameter of the inhibitory zone surrounding the disks was measured with the aid of caliper, The isolate sensitivity/resistance pattern was examined using the reference standard by the Clinical Laboratory Standards Institute [18]. Antibiotics discs used were gentamicin (10 µg), amoxicillin (25 µg) augmentin (30 µg), nitrofurantoin (30 µg), erythromycin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), ofloxacin (5 µg), ceftriaxone (30 µg),ciprofloxacin (5 µg) and vancomycin (30 µg). They were tested against all *S. aureus* and MRSA isolates. *Staphylococcus aureus* (ATCC 25923) was used as control. Methicillin Resistance *Staphylococcus aureus* was determined using cefoxitin discs on Muella-Hinton agar.

3. RESULTS AND DISCUSSION

Urinary tract infections in pregnancy is a potentially life threatening condition and despite improved treatment and advances in diagnosis, bacterial urinary tract infection remains one of the major cause of morbidity and mortality during pregnancy worldwide [19]. Variation in urinary tract infections causative agents and their antibiotic susceptibility patterns from time to time make it important for the continuous surveillance to provide information on the appropriate control measures and antibiotics policy in a particular environment [2].

Out of the total samples screened 56 samples (6.5%) showed significant *Staphylococcus aureus* bacteriuria with the prevalence rate varied from 5% at Sobi Specialist Hospital to 8.3% at Adewole Cottage Hospital. This result is comparable with results from other places such as: Muhammed, [20] isolated 9 (18%) out of 50 samples screened; Onoh et al. [5] reported 52 (9.6%) was isolated from 542 pregnant women in Abakaliki; Ajayi et al. [21] reported 28.8% out of 125 samples screened at UITH; 10.4% urinary tract infection was reported at University of Gonder Teaching Hospital, Ethiopia [22]; 14.6% in Tanzania; 25% reported by Lawani et al. [23] among pregnant women from Southern Nigeria; Fred et al. [5] reported 26.7% in Nairobi and 55% was reported by Oladeinde et al. [24] in Benin City, Nigeria [21,22,23,20,24,5]. Furthermore the prevalence is lower compare to bacteriuria reported by Zahra Tayebi et al. [25] and Singh et al. [26] in India.

Table 1. Distribution of *Staphylococcus aureus* and methicillin resistant *S. aureus* detected among the pregnant women in selected hospitals in Ilorin Kwara State

Hospital	Total sample	*S. aureus*	MRSA
Sobi	383	19	3
Adewole	278	23	2
Ajikobi	195	14	2
Total	856	56	7

Table 2. Age distribution of *S. aureus* bacteriuria in pregnant women in all selected hospitals

Age	Total-sample collected			*S. aureus* No (%)			MRSA		
(year)	SOBI	ADEWOLE	AJIKOBI	SOBI	ADEWOLE	AJIKOBI	SOBI	ADEWOLE	AJIKOBI
15-25	138	102	63	11	4	2	1	-	1
26-35	182	131	104	6	10	8	2	2	1`
35-45	63	45	28	8	5	2	-	-	-

Percentage

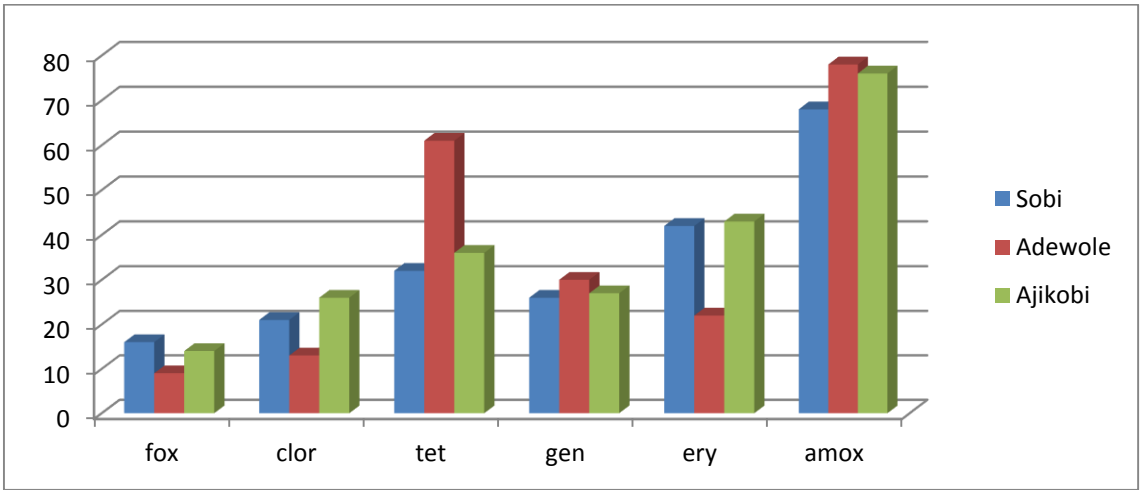

Fig. 1A. Resistant profile of *Staphylococcus aureus* bacteriuria in pregnant women in selected hospitals compared in Ilorin Nigeria

Percentage

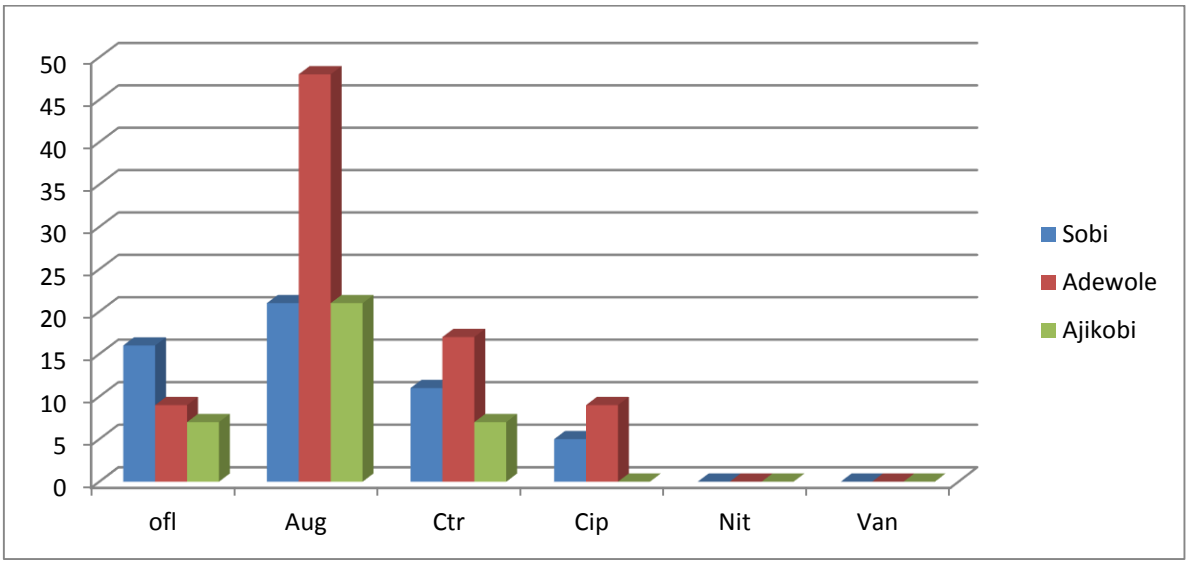

Fig. 1B. Resistant profile of *Staphylococcus aureus* bacteriuria in pregnant women in selected hospitals in Ilorin Nigeria

Key FOX= Cefoxitin, CLOR=Chloramphenicol, GEN=gentamicin, ERY= erythromycin, OFL=ofloxacin; CIPRO= Ciprofloxacin AUG= Augmentin, CTR= ceftriaxone, TET= Tetracycline, Nit= nitrofurantoin, Van= vancomycin

Major variations in prevalence of urinary tract infections could be explained by pregnancy associated physiological changes, differences in socio-economic, educational level, personal and environmental hygiene [27,19]. Many other factors such as genetic make-up of the studied populations, higher anal colonization, poor anal hygiene could lead to contamination of urinary tracts through vagina and religion as majority of the patients in this study were Muslims that were used to frequently washing of external genitalia after urination.

The study also showed that pregnant women between the age range of 35-45 years have the highest prevalence rate of 10. 9% while the women in the range of 15-25years have the lowest rate with 5.6%. This is in disagreement with a study by Buzayan in Libya who reported highest prevalence among women with 25-30 years [28].

Previous studies have also shown that more than 90% of urinary tract infections are caused by microbes that are normal floral that consequently contaminate the external genitals and invade the urinary tract [2, 29]. Many factors are responsible to high prevalence of urinary such as indiscriminate antimicrobial usage, incomplete treatment for bacteriuria by untrained personnel, or treatment of other bacterial infections. It is also possible that some of these groups could have had a previous hospitalization.

In this investigation, the overall prevalence of MRSA among isolated *S. aureus* is 12.5%, this is lower compare to earlier studies conducted in Iran (25.8%), Nepal (30.8%), Uganda (35%) and 27.9% in Ireland [31], Bahati et al. [30]. Mitiku et al. [31] also reported 23 (43.4%) MRSA isolates among 53 S. aureus isolates in a study in in Ethiopia, which was slightly higher than 44.8 % recorded the study conducted in India [32]. This difference in MRSA isolation rates could be due to the isolation technique (multiple screens), sample collection method (usage of more than one clinical specimen), and variation in study participants.

Antibiotic sensitivity against the isolated *Staphylococcus aureus* shows that 19.6% resistance to Chloramphenicol was recorded, tetracycline has 44.6% resistance, gentamicin has 14.3% resistance, erythromycin (33.9%), amoxicillin (67.9%), ofloxacin (10.7%), augmentin (32%), ceftriaxone (12.5%) while 100% susceptibility to nitrofurantoin and vancomycin was recorded. Therefore nitrofurantoin, ofloxacin, ceftriaxone and gentamicin showed highest sensitivity in this

study. Nitrofurantoin is one of the safest drug during pregnancy and this sensitivity to nitrofurantoin is in agreement with 85.7% reported by Oluwafemi et al. [33] in tertiary health in South West Nigeria. Fluoroquinolones is a major group of antibiotics widely used in the empirical treatment of UTI and ofloxacin have also found to be one of the most sensitive and effective antibiotics against uropathogen. High resistance to amoxicilin and augmentin may be due to over the counter availability of these drugs and increase in the abuse of these drugs which could lead to mutations that could promote resistance through plasmid or bacteriophage mechanism.

5. CONCLUSIONS AND RECOMMENDATIONS

Detection of MRSA in the pregnant women highlightened the need for the proper screening of all pregnant women in order to avoid maternal chorioamnionitis, intrauterine growth retardation, premature rupture of membrane, and low birth weight associated with bacteriuria. Early detection and prompt treatment with appropriate antibiotics will reduce and prevent associated complications. Urinary culture and sensitivity is also recommended to be the gold standard in diagnosing urinary tract infection.

ETHICAL APPROVAL AND CONSENT

Ethical approval was obtained from Kwara State Ministry of Health and KWASU Center for Community Development. Only consented asymptomatic pregnant women were included in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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