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Evaluation of the Prevalence, Antimicrobial Resistance Trait, and Virulence Determinants in *Staphylococcus aureus* Isolates from the Anogenital Area of 35-37 Weeks Pregnant Women

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Abstract

Staphylococcus aureus is a major pathogen in the postpartum period being frequently implicated as a cause of mastitis, and nursery outbreaks, among others as well as its increasing resistance to common antibiotics. This study therefore aimed at evaluating the presence of *S. aureus* in pregnant women attending clinics in rural towns in Eastern Cape, South Africa. A total of 82 pregnant women at 35–37 weeks of gestation were sampled for the presence of *S. aureus*. Using standard microbiological methods, presumptive *S. aureus* isolates were obtained from vaginal and rectal swab samples. The isolates were confirmed by conventional polymerase chain reaction and then tested for antimicrobial susceptibility using the disc diffusion technique. Presence of resistance genes and virulence determinants were equally profiled. A total of 47 (57.3%) out of 82 pregnant women were colonized with *S. aureus*. Only two virulence genes *PVL*- 37/47 (78.7%) and *eta*-10/47 (21.3%) were detected in the isolates. The isolates were all resistant to penicillin G and clindamycin (100%), while resistances to tetracycline, vancomycin, rifampicin were 71% and resistance to oxacillin and erythromycin were above 80% while resistance to other antibiotics tested were below 40%. The isolates showed multiple resistance to 5-6 antimicrobials with indices ranging from 0.5-0.6. Genes encoding resistance to erythromycin (*erm*B), tetracycline (*tet*M), and rifampicin (*rPOB*) were found in 72.7% (34/47) of the isolates, while 15% (7/47) possessed the *Bla-Z* (penicillin). The high antibiotics resistance traits found in the isolates analysed indicates limited therapeutic options and portents a major threat to public health.

Keywords: *Staphylococcus aureus*, virulence genes, antimicrobial resistance, pregnant women

Резюме

Staphylococcus aureus е основен патоген в следродилния период. Той често се споменава като причина за мастит и огнища в детските градини, наред с нарастващата му резистентност към обичайните антибиотици. Ето защо, това проучване има за цел да оцени наличието на *S. aureus* при бременни жени, посещаващи клиники в селски градове в Източен Кейп, Южна Африка. От общо 82 бременни жени в 35-37-ата гестационна седмица бяха взети проби за наличие на *S. aureus*. С помощта на стандартни микробиологични методи бяха получени предполагаеми изолати на *S. aureus* от проби от вагинален и ректален тампон. Изолатите бяха потвърдени чрез конвенционална полимеразно-верижна реакция и след това бяха изследвани за антимикробна чувствителност с помощта на дисково-дифузионна техника. Наличието на гени за резистентност и детерминанти на вирулентност бяха еднакво профилирани. Общо 47 (57.3%) от 82 бременни жени бяха колонизирани със *S. aureus*. В изолатите бяха открити само два гена за вирулентност PVL- 37/47 (78.7%) и eta-10/47 (21.3%). Всички изолати бяха резистентни към пеницилин G и клиндамицин (100%), докато резистентността към тетрациклин, ванкомицин, рифампицин беше 71%, а резистентността към оксацилин и еритромицин беше над 80%, а към другите тествани антибиотици беше под 40%. Изолатите показаха множествена резистентност към 5-6 антимикробни средства с индекси, вариращи от 0.5-0.6. Гени, кодиращи резистентност към еритромицин (ermB), тетрациклин (tetM) и рифампицин (rPOB), бяха открити при

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72.7% (34/47) от изолатите, докато 15% (7/47) притежаваха Bla-Z (пеницилин). Високите белези на антибиотична резистентност, открити в анализираните изолати, показват ограничени терапевтични възможности и предвещават сериозна заплаха за общественото здраве.

Introduction

Vaginal microbiota, which is a varied community of microorganisms colonizing the human vagina, can affect the health of women, their partners, and neonates (Shi *et al.,* 2016; Yeta *et al.,* 2021). It has been asserted that colonization of the vagina with pathogenic bacteria and the resultant susceptibility of women to urinary tract infections (UTI) occur mainly due to factors including pregnancy, short urethra, lack of prostatic secretion, and easy contamination of the female urinary tract with faecal floral (Demilie *et al.,* 2012; Yeta *et al.,* 2021); and the pathogens could be easily transmitted to the baby, especially during complicated labour and delivery (Li *et al.,* 2021; Tumuhamye et al., 2021). Many microbial agents have been implicated as common causative agents of UTIs, however, the *Escherichia coli*, *Staphylococcus aureus,* and some other staphylococci are known to be cogent pathogenic bacterial strains implicated in many human reproductive diseases (Tumuhamye *et al.,* 2021; Pellati *et al.,* 2008; Dutta *et al.,* 2020). Notably, *S. aureus* is counted among the abnormal vaginal flora such as *E. coli*, *Trichomonas vaginalis*, and streptococci, that contribute immensely to the oncoming of aerobic vaginitis, one of the clinical conditions that ensue due to abnormal flora in the vagina (Sengupta *et al.,* 2020; Tesfaye *et al.,* 2022). *S. aureus* has evolved as a normal flora because of its evolution in the host, allowing it to adapt to the environment and interact with biotic and abiotic factors. Its characteristics, such as the thick peptidoglycan cell wall, allow it to live and grow at high salt concentrations. It preferably colonizes the dark and moist skin areas with the anterior nares being a common site of colonization. The throat, groin, and axillae are, however, the major reservoir of *S. aureus* (Wasserman and Taljaard 2010). *S. aureus* can infect all the tissues of the body, resulting in diseases that can range from small skin conditions to fatal cases like toxic shock syndrome. *S. aureus* outbreaks have been reported in paediatrics since the 1800s (Dancer *et al.,* 1990; Popoola and Milstone, 2014; Becker and Wardenburg, 2015), and ever since, it has continued to be reported as a major cause of outbreaks in health-care-associated infections (HAIs), especially in neonatal intensive care units (NICUs) globally (Gastmeier *et al.,* 2007; Hocevar *et al.,* 2012). *S. aureus* is a significant source of healthcare-associated disease, with an estimated 23.8 per 1000 hospitalisations in the United States of America in 2005 (Lin and Peterson, 2010). Hospital patients that have gone through surgery are in danger of contracting the organism with a resultant disease, as colonization often results in hospital and community disease outbreaks (von Eiff *et al.,* 2001; Wertheim *et al,* 2004).

An estimate of 20% of the human populace is frequently colonized by *S. aureus* with 60% carrying it irregularly while 20% do not carry *S. aureus,* and 4-22% of pregnant women have vaginal colonization (Beigi and Hanrahan, 2007; Creech *et al.,* 2010; Hetsa *et al.,* 2018). In a study conducted by Top *et al.* (2010), young age and GBS colonization of anogenital correlated with colonization by *S. aureus*, however, the demographic or maternal factors were not explored. The two major risk factors observed for *S. aureus* colonization in Africans are HIV infection and immunodeficiency associated with genetic factors (Peacock *et al.,* 1999; Olalekan *et al.,*2012; Kinabo *et al.,* 2013; Hetsa *et al.,* 2018).

Although *S. aureus* is known to be non-invasive, evidence has shown that the bacterial pathogen can colonize varieties of host cells through an efficient mechanism of fibronectin bridge formation between the fibronectin-binding proteins in the bacterium and the host's integrin molecules, thereby triggering internalization (Schwarz-Linek *et al.,* 2004). An array of virulence factors is produced to facilitate its pathogenicity, namely, haemolysin, leukocidins, protease, enterotoxins, exfoliative toxins, and immunomodulatory factors (Manders, 1998; Foster, 2004; Foster, 2005; Denis *et al.,* 2005; Rooijakkers *et al.,* 2005), and their expression is regulated by the process of growth. Possession of some virulence factors is known to correlate with certain infections such as Panton-Valentine leukocidin (*PVL*), toxic shock syndrome (TSS), necrotizing pneumonia, dermal infections by exfoliative toxins A and B (ETA and ETB) and scalded skin syndrome. Others include atopic dermatitis, impetigo, the family of staphylococcal enterotoxins A and B (SEA and SEB), and food poisoning.

Many toxins constitute the virulence determinants that can manipulate the components of the immune response, including leucotoxins, which are known to affect white blood cells (WBC), as well as an extensive scalding of the dermis, toxins functioning as superantigens that engender the immune system to release cytokines excessively (SEA,

SEB, and TSST), and haemolysins that are potent in lysing erythrocytes and various WBCs, as well as other types of cell. Reports on severe pneumonia and complex skin and soft tissue infections in Africans are often related to *S. aureus* strains producing the PVL (von Eiff *et al.,* 2004; Danis *et al.,* 2005; Beilouny *et al.,* 2008; Braurec *et al.,* 2011; Zanger *et al.,* 2012). The African region is currently known as endemic for *PVL* isolates, having a high prevalence ranging from 17% to 74% (Breurec *et al.,* 2011a]; unlike the prevalence of 9%–14% observed in Europe (von Eiff *et al.,* 2004).

The ability to acquire antibiotic resistance is an important factor that enables *S. aureus* to successfully evolve as a pathogen (Otto, 2012; Watkins *et al.,* 2012). An interesting feature of *S. aureus* in urban places is their reported abnormal level of resistance to some antibiotics such as penicillin with about 73.7–100% (Ramdani-Bouguessa *et al.,* 2006; Kolawole *et al.,* 2013), co-trimoxazole with 15-89% (Mariem *et al.,* 2013), and other antibiotic medications with resistance prevalence ranging from 21.8-92% (Vergison *et al.,* 2012; Conceiçao *et al.,* 2014). It is interesting to know that this is contrary to the observable lower prevalence of the resistant strains in people that are from remote areas, with recorded 35.3% resistance to penicillin, 11.8% to co-trimoxazole, and 5.8% to tetracycline (Kolawole *et al.,* 2013; Mariem *et al.,* 2013; Conceiçao *et al.,* 2014).

Urinary tract infections during pregnancy have become a common health trouble in developed and developing countries of the world, especially with the continual emergence of antimicrobial-resistant pathogens. A substantial increment in the occurrence of UTIs among pregnant women has been observed globally, and it is a known cause of morbidity and negative pregnancy outcomes (Karambo and Africa, 2017). The occurrence of antimicrobial-resistant uropathogenic bacteria such as *S. aureus* in pregnant women, especially in developing countries, has necessitated a recent call for the incorporation of culture and antimicrobial drugs susceptibility testing into the usual antepartum care for pregnant women and also the need for monitoring drug resistance (Karambo and Africa, 2017). In South Africa and some other Southern African countries, some significant efforts have been made and reported on monitoring the prevalence and resistance of *S. aureus* and other uropathogenic bacteria in pregnant women with their clinical outcomes (Madhi *et al.,* 2019; Nana *et al.,* 2021; Zwane *et al.,* 2021; Orji *et al.,* 2022). However, to the best of our knowledge, there is a paucity of information on the prevalence and drug resistance profile of *S. aureus* in pregnant women in Amatole and Buffalo City District Municipalities of Eastern Cape, South Africa. This study was therefore designed to unravel the prevalence, drug resistance characteristics, and occurrence of virulence determinants in *S. aureus* isolates from pregnant women in the study area.

Methods and Materials

Ethical consideration

The ethical approval to carry out this study and permission to collect samples from health facilities in the study areas were sourced from the University of Fort Hare (Alice, South Africa) Ethics Committee and the Eastern Cape Department of Health respectively. Ethical issues such as strict confidentiality and informed consent were adhered to. The involvement in the study was entirely voluntary and the study was explained to participants who were at 35 to 37 weeks of pregnancy before the enrolment.

Study area and sample collection

A once-off sampling was done, and one hundred and sixty-four (164) rectovaginal swabs were collected from some selected clinics within the Eastern Cape. The samples were collected at Victoria hospital, Dimbaza 1 and 2, War memorial, Middle drift, and Mdantsane NU (12, 13, and 17) clinics, within the Amathole and Buffalo City district municipalities, from asymptomatic pregnant women at 35–37 gestation, by qualified healthcare personnel and were carried on ice packs to the Microbiology laboratory facility at the University of Fort Hare.

Detection and isolation of Staphylococcus aureus

For detection and isolation of *S. aureus*, samples were aseptically inoculated into a Todd Hewitt broth (fortified with gentamycin, colistin, and nalidixic acid) and subjected to incubation at 37°C for 18-24 hours under 5% CO_2. Following the incubation process, the bacterial cultures were sub-cultured onto a Mannitol salt agar (MSA) plate and cultured at 37°C for 18–24 hours under 5% CO_2. After incubation, the plates were inspected for the presence of colonies suggestive of *S. aureus*. Pure colonies were picked and inoculated into a trypticase soy broth (TSB) and incubated at 37°C for 24 h. Glycerol stock was prepared from the broth culture and preserved at -80°C for further analysis.

Extraction of genomic DNA from the isolates

The bacterial isolates were resuscitated from the glycerol stocks by growing them overnight in

fresh TSB at 37°C from the culture. The previously described boiling method of Torres *et al.* (2003) was adopted for the extraction of DNA. The purified colony of each isolate was picked aseptically and suspended in a 200 µL sterile distilled water and boiled for 15 minutes at 100°C. The centrifugation of the suspension was done at 10,000 rpm for 10 minutes, the supernatant was transferred into a sterile Eppendorf tube and the pellet was discarded. The supernatant representing the DNA (template) of the isolate was stored at -20°C for further analysis.

Molecular characterization

Confirmation of isolates through screening for the Nuc gene

A 5 µL template was amplified in a 25 µL reaction mixture consisting of 12 µL of 2× PCR master mix (Thermo Scientific, SA), 1 µL each of both forward and reverse *nuc* primers, and 6 µL of PCR-grade water (Thermo Scientific, SA). The thermal cycler conditions used include the first denaturation step at 94 °C for 4 min, followed by 40 cycles of final denaturation at 94 °C for 60 sec, an annealing step at 58 °C for 60 sec, and an initial extension step at 72 °C for 60 sec; the final extension step was carried out at 72°C for 7 min. Amplicons obtained were analysed in 1.5% agarose gel stained with ethidium bromide, while a 0.5X TBE was used as a running buffer. The electrophoresed gel was viewed and documented using an Alliance 4.7 UV-transilluminator (Uvitec, Cambridge, United Kingdom).

Screening for virulence genes

The screening of virulence genes was performed with a singleplex endpoint PCR method, for *eta, etb, pvl, hla, hlb, hld, LUKM*, and *LUKED* with primer sequences as shown Table 1. A 5 µL template was amplified in a 25 µL reaction mixture consisting of 2 × PCR master mix (Thermo Scientific, SA), 1 µL each of both forward and reverse primers specific for highlighted virulence genes, and 6 µL of PCR-grade water (Thermo Scientific, SA). The thermal cycler conditions used consist of first denaturation at step 94 °C for 3 min, 40 cycles of final denaturation at 94 °C for 90 sec, annealing step at 58 °C for 60 sec, and initial extension step at 72 °C for 90 sec; followed by the final extension at 72 °C for 7 min. Amplicons obtained were analysed in 1.5% agarose gel stained with ethidium bromide, while a 0.5X TBE was used as a running buffer. The electrophoresed gel was viewed and documented using an Alliance 4.7 UV-transilluminator (Uvitec, Cambridge, United Kingdom).

Antimicrobial susceptibility testing

The antibiotic susceptibility testing of the confirmed *S. aureus* isolates was carried out using the disc diffusion method on Mueller Hinton agar plates (Bauer *et al.,* 1966)], following the Clinical Laboratory Standard Institute guidelines (CLSI, 2016). Briefly, to prepare the inoculum for the assay, pure colonies of isolates were taken aseptically from 18

Table 1. List of primers used in the study

Target gene	Primer Sequences 5'-3'	Amplicon size (bp)	Reference
Nuc	*Nuc* Forward - GCG ATT GAT GGT GAT ACG GTT *Nuc* Reverse - ACG CAA GCC TTG ACG AAC TAA AGC	279	(Brakstad *et al.,* 1992)
Hla	*hla* Forward - CTGATTACTATCCAAGAAATTCGATTG *hla* Reverse – CTTTCCAGCCTACTTTTTTATCAGT	209	(Jarraund *et al.,* 2002)
Hlb	*hlb* Forward – GTGCACTTACTGACAATAGTGC *HLB* Reverse - GTTGATGAGTAGCTACCTTCAGT	309	(Jarraund *et al.,* 2002)
Hld	*hld* Forward -AAGAATTTTTATCTTAATTAAGGAAGGAGTG *hld* Reverse - TTAGTGAATTTGTTCACTGTGTCGA	111	(Jarraund *et al.,* 2002)
LUKM	*LUKM* Forward – TGGATGTTACCTATGCAACCTAC *LUKM* Reverse - GTTCGTTTCCATATAATGAATCACTAC	780	(Jarraund *et al.,* 2002)
LUKED	*LUKED* Forward - TGAAAAAGGTTCAAAGTTGATACGAG *LUKED* Reverse - GTATTCGATAGCAAAAGCAGTGC	269	(Jarraund *et al.,* 2002)
PVL	*PVL* Forward – GAGGCCAATCGTTGCACGTAA *PVL* Reverse – AACCTTCTCCTTCACACTAATCCT	701	(Jarraund *et al.,* 2002)
Eta	*eta* Forward – ACTGTAGGAGCTAGTGCATTTGT eta Reverse – TGGATACTTTTGTCTATCTTTTTCATCAAC	190	(Jarraund *et al.,* 2002)
Etb	etb Forward - CAGATAAAGAGCTTTATACACACATTAC etb Reverse - AGTGAACTTATCTTTCTATTGAAAAACACTC	612	(Jarraund *et al.,* 2002)

to 24 h cultures plated on Nutrient Agar (Himedia Laboratories) and used to make suspension (with turbidity equivalent to 0.5 McFarland standard) in 2 mL of sterile normal saline solution (0.9%). Aseptically, sterile swab sticks were inserted into the inoculum tubes, drained by pressing against the test tube wall, and used to streak the dried surface of Mueller Hinton agar plates appropriately. The streaked plates were left to sit at normal room temperature for about 3 to 5 min to allow the absorption of the inoculum into the agar before placing the antibiotic discs. The dispenser was placed over the agar plates and the plunger was firmly pressed to dispense the discs onto the surface of the seeded agar. The plates were incubated at 37°C for 18 h and zones of inhibition were recorded and interpreted according to the CLSI standard [56]. The antibiotics used include: cefuroxime (30 μg), tetracycline (30 μg), rifampin (5 μg), erythromycin (15 μg), imipenem (10 μg), clindamycin (2 μg), penicillin G (10 μg), vancomycin (30 μg), norfloxacin (10 μg), sulfamethoxazole-trimethoprim (300 μg), gentamycin (10 μg) and chloramphenicol (30 μg). All the "intermediate resistant" isolates were taken as "resistant" isolates. For the resistant isolates, multiple antibiotic resistance (MAR) phenotyping and indexing were done. The MAR index (MARI) of individual isolate in each serogroup identified was determined by using the formula described by Titilawo *et al*. (2020) as follows:

$$\text{MAR index of isolate} = \frac{\text{No. of antibiotics to which an isolate showed resistance}}{\text{Total no. of antibiotics to which an isolate was exposed}}$$

Antimicrobial resistance gene determination

Possession of relevant antimicrobial resistance genes by the isolates was determined by screening for some selected genes including *ermB* for erythromycin resistance, *tetM* gene which encodes for tetracycline resistance, *vanB* which encodes for vancomycin resistance, *linB* which encodes for clindamycin resistance, and *bla-Z* that codes for beta-lactamase (penicillin G) resistance, using primer sequences shown in Table 2. The PCR reaction mixture of 25 μL volume was used comprising 12 μL of 2× PCR master-mix (Thermo Scientific, SA), 6 μL of PCR-grade water (Thermo Scientific, SA), 1 μL each of both the forward and reverse primers for each resistance gene tested, and lastly, 5 μL of the extracted DNA template.

Results

S. aureus isolation

A sum total of one hundred and sixty-four (164) samples were obtained from the vaginal and rectal swabs of 82 pregnant women from Dimbaza 1, War memorial, Middle drift, Mdantsane NU12 communities in the Eastern Cape Province, South Africa. Sequel to preliminary screening on culture medium, a total of sixty-two (62) presumptive isolates were obtained using the mannitol salt agar, a selective medium that increased the chances of detecting staphylococcal species by inhibiting the growth of other organisms.

Molecular confirmation of S. aureus isolates

The outcome of the molecular confirmation of the presumptive *S. aureus* isolates showed that 47/62 (75.8%) isolates came out positive for the *nuc* gene used for the confirmation, out of which 38 (80.6%) were from vaginal samples and 9 (19.4%) from rectal samples (Fig. 1).

Molecular virulence gene typing of the S. aureus isolates

The forty-seven (47) confirmed *S. aureus* iso-

Table 2. Primers for profiling resistance genes

Target gene	Primer sequence 5' to 3'	Amplicon size (bp)
ErmB	*ErmB* Forward – CTATCTGATTGTTGAAGAAGGATT *ErmB* Reverse – GTTTACTCTTGGTTTAGGATGAAA	142
TetM	*TetM* Forward – AGTGGAGCGATTACAGAA *TetM* Reverse – CATATGTCCTGGCGTGTCTA	158
Bla-Z	*Bla-Z* Forward - ACTTCAACACCTGCTGCTTTC *Bla-Z* Reverse – TGACCACTTTTATCAGCAACC	173
VanA	*VanA* Forward - GTTGCAATACTGTTTGGGGG *VanA* Reverse – CCCCTTTAACGCTAATACGATCAA	1014
VanB	*VanB* Forward - GTGACAAACCGGAGGCGAGGA *VanB* Reverse – CCGCCATCCTCCTGCAAAAAA	484
RpoB	*rpoB* Forward – ACCGTCGTTTACGTTCTGTA *rpoB* Reverse – TCAGTGATAGCATGTGTATC	460

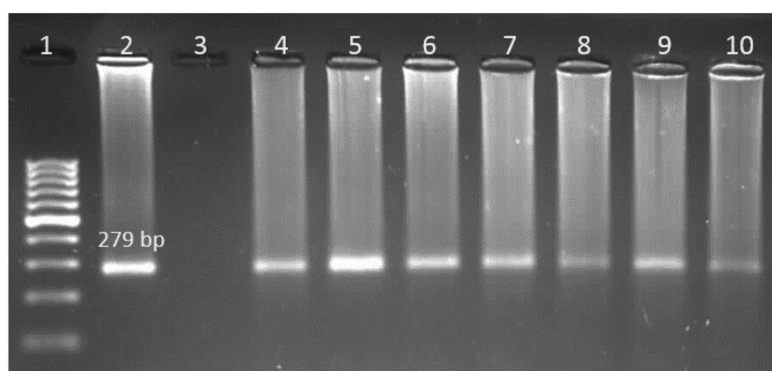

Fig. 1. PCR products for *S. aureus* confirmation based on *nuc* gene amplification. Lane 1: 100bp DNA ladder, Lane 2: positive control, Lane 3: negative control, Lane 4-10: positive isolates.

lates were further examined for the possession of virulence genes. It was revealed that 21.3% (n=10) harboured *eta*, while 78.7% (n=37) possessed the *PVL* (Table 3).

Table 3. Percentage occurrences of *S. aureus* virulence genes among the recovered isolates

Virulence	No. of isolates N (%)
ETA	10 (21.3%)
ETA	0 (0%)
HLA	0 (0%)
HLB	0 (0%)
HLD	0 (0%)
LUKM	0 (0%)
LUKED	0 (0%)
PVL	37 (78.7%)
TST	0 (0%)

Antimicrobial resistance pattern of the S. aureus isolates

The antimicrobial resistance distribution of the *S. aureus* isolates is shown in Table 4 and Fig. 2. Though the isolates showed varying degrees of susceptibility to the antimicrobial agents tested, however, a hundred percent (100%) resistance was exhibited against penicillin G and clindamycin. This was followed by 71.4% (5/7) resistance of the isolates to tetracycline, rifampicin, oxacillin, and erythromycin. Resistance to norfloxacin, gentamycin, and sulfamethoxazole-trimethoprim was found in 42.9% (3/7) isolates, while only one isolate (representing 14.3%) was resistant to chloramphenicol.

Multiple antibiotic resistance phenotypes (MARP) and indices (MARI)

The results of the MARP and MARI for the *S. aureus* isolates with *PVL* and *eta* genes are expressed in Tables 5 and 6 respectively. All the

Table 4. Result for antimicrobial susceptibility profiling of *S. aureus* isolates

Antibiotics	Code	Potency (µg)	R n (%)	S n (%)
Penicillin	PG	10	47(100%)	0 (0%)
Vancomycin	VA	30	37 (71.4%)	10 (28.6%)
Clindamycin	CD	2	47 (100%)	0 (0%)
Tetracycline	T	30	37 (71.4%)	10 (28.6%)
Rifampicin	RP	5	37 (71.4%)	10 (28.6%)
Imipenem	IMI	10	6 (12.7%)	41 (87.2%)
Gentamycin	G	10	18 (38.2%)	29 (61.7%)
Chloramphenicol	C	30	16 (34.0%)	31 (66%)
Norfloxacin	NOR	30	19 (40.4%)	28 (59.6%)
Oxacillin	OX	10	39 (83%)	8 (17.0%)
Erythromycin	E	15	40 (85%)	7 (15%)
Sulfamethoxazole-trimethoprim	SXT	30	19 (40%)	28 (60%)

R= resistant, S= Susceptible P: Penicillin G, VA: Vancomycin, T: Tetracycline, CD: Clindamycin, RP: Rifampicin, IMI: Imipenem, G: Gentamycin, C: Chloramphenicol, OX: Oxacillin, E: Erythromycin, NOR: Norfloxacin, SXT: Sulfamethoxazole-Trimethoprim.

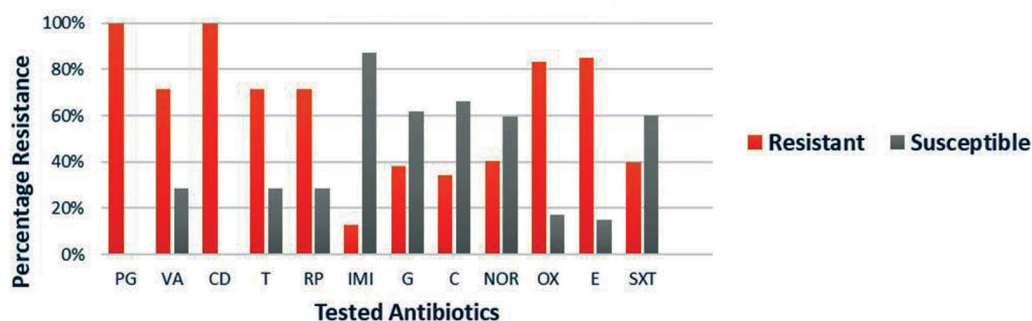

Fig. 2. Antimicrobial susceptibility pattern of *S. aureus* isolates
R= resistant, S= Susceptible P: Penicillin G, VA: Vancomycin, T: Tetracycline, CD: Clindamycin, RP: Rifampicin, IMI: Imipenem, G: Gentamycin, C: Chloramphenicol, OX: Oxacillin, E: Erythromycin, NOR: Norfloxacin, SXT: Sulfamethoxazole-Trimethoprim.

isolates showed marked resistance to at least 3 antibiotics, while multiple antibiotic resistance was observed among the isolates against various antibiotics ranging from 4 to 11. The predominant MARP was PG-VA-T-CD-OX-E-RP-G, while the MARI for the isolates ranged between 0.7 - 0.8, with an average of 0.7.

Table 5. Multiple antibiotic resistance (MAR) phenotypes and indices (MARI) of *S. aureus* isolates with *PVL* gene.

MAR phenotype	Number of antibiotics	MARI
PG-VA-T-CD-OX-E-RP-G	8	0.7
PG-T-CD-OX-E-RP-SXT-C	8	0.7
PG-T-CD-RP-E-SXT-VA	7	0.6
PG-T-CD-RP-OX-E-NOR-IMI	7	0.6
PG-VA-T-CD-OX-E-RP-C	8	0.7
PG-VA-G-CD-NOR-RP-E	7	0.6

Table 6. Multiple antibiotic resistance (MAR) phenotypes and indices (MARI) of *S. aureus* isolates with *eta* gene.

MAR phenotype	Number of antibiotics	MARI
PG-CD-VA-NOR-SXT-RP-C	7	0.6

Molecular characterization of the S. aureus isolates based on the presence of resistant genes

The confirmed *S. aureus* isolates came out positive for various resistance genes as follow: *tetM* – 34/47 (72.3%), *ermB* – 34/47 (72.3%), *bla-Z* – 7/47 (15 %), and *rpoB* – 34/47 (72.3%) while, none of the isolates came out positive for *vanA* and *vanB* antibiotics resistance genes profiled.

Discussion

Globally, *S. aureus* is known to be a common pathogen responsible for various infections of humans for a long time. Numerous studies identify the organism as a causative agent in different infections states and the common sources of colonization include health care workers' hands, and poor hygiene due to inadequate disinfection of the environment to mention but a few (Popoola and Milstone, 2014]. This empirical study was conducted to check for the occurrence of *S. aureus*, characterize the virulence factors, profile antimicrobials resistance pattern, and resistance determinants in *S. aureus* isolates recovered from the recto-vaginal area of selected pregnant women at the gestation period of 35 to 37 within the Province. The presence of virulence genes in the isolates was evaluated to assess their pathogenicity potential.

In the study, we identified contradicting information from the results of the selective media, Mannitol Salt Agar (MSA) that depicted the presumptive colonies of the *S. aureus*. Most of the presumptive isolates could not be ascertained as *S. aureus* when molecular confirmation was performed using specific primers reported in the literature. Approximately 57.3% of the pregnant women sampled were colonised with *S. aureus*, with 57% obtained from the vaginal swabs, and 43% obtained from the rectum. This is higher than the colonisation rates reported in some literature where 14.5% and 17.1% were recorded in pregnant women (Chen *et al.,* 2006; Andrew *et al.,* 2008). It has been evident that *S. aureus* colonisation and its associated diseases have been increasing in pregnant women and postpartum women, as well as in healthy infants and neonates in the hospital intensive care units (Top *et al.,* 2010). Although the colonisation rate recorded here is relatively lower than in previous studies, the resultant colonisation of the neonates through their mothers, which can lead to infections such as sepsis meningitis and pneumonia, makes any level of colonisation of *S. aureus* in the anogenital area a serious concern.

The virulence genes detected among the study isolates showed that *PVL* (78.7%) was the predominant virulence gene, followed by *eta* at 21.3%. In-

creased reports of *PVL*-positive isolates have made it endemic in Africa (Okon *et al.,* 2009; Breurec *et al.,* 2011; Schaumburg *et al.,* 2011a; Schaumburg *et al.,* 2011b). The high prevalence of *PVL*-positive isolates in the community enables the isolate to spreads in hospitals, particularly in Africa, because the family and friends are always present in hospitals, and some healthcare responsibilities are transferred to these individuals thus increasing the chances of pathogen circulating between the community and hospital settings (Breurec *et al.,* 2011b).

Notably in this study, the totality of *S. aureus* isolates tested exhibited resistance to clindamycin and penicillin, while high resistance was shown to tetracycline, erythromycin, rifampicin, oxacillin, gentamycin, chloramphenicol, norfloxacin, sulfamethoxazole-trimethoprim, and imipenem. The 71.4% resistance rate exhibited to vancomycin and 38% to gentamycin were far much higher than the 18% and 17% resistance reported respectively against the drugs in another study by Abdulrazaq *et al.* (2014) in Baghdad. A large proportion of *S. aureus* isolates from this study exhibited multiple antimicrobial resistance, with the most dominant MAR pattern being PG-VA-T-CD-OX-E-RP-G, which occurred among isolates harbouring *PVL* virulence gene. Overall, the MAR index of 0.7 to 0.8 was recorded in this study with an average value of 0.7. This finding supports the belief that extensive and indiscriminate usage of antibiotics could be a root cause of the high risk of multidrug-resistant organisms in humans.

Erythromycin, rifampicin, and tetracycline resistance were found to be dominant due to the presence of the *erm*B methylase, rpoB, and *tet*M genes that code for the observed resistance amongst the isolates respectively. The majority of the isolates, precisely 72.3%, harbour resistance genes including *erm*B methylase, rpoB, and *tet*M, while only 7 (15%) was found to harbour the *bla-Z* gene. However, *van*A and v*an*B which encode resistance against vancomycin were not detected. In the remaining isolates even though resistant to the antibiotics tested, no resistance genes were detected in them using conventional PCR. This observation further stressed the probability that other mechanisms may also be involved in the process.

Conclusion

The *S. aureus* colonisation in pregnant women as observed in this study is relatively low, however, it is an issue of serious concern due to the severe outcomes it could have for neonates and infants. The distribution of virulence genes showed the presence of *PVL* and *eta,* which are commonly found in the skin. The phenotypic and genotypic resistance profile depicting multi-antibiotic drug resistance was observed in the isolates thus indicating an overuse and misuse of antibiotics. This requires more stringent regulations on the usage of antibiotics among the populace to stem the ugly tide of drug resistance among pathogenic bacteria of clinical importance. Further studies should be done to fully elucidate the prevalence *of S. aureus* colonisation and their antibiogram profiles to generate more data of epidemiological importance in the study area and across the country.

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