Vaginal colonization with virulent and methicillin resistant *Staphylococcus aureus* among Ugandan women in Labour

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Abstract

Background *Staphylococcus aureus* (*S. aureus*) often colonizes the human skin, upper respiratory and genital tracts. In the female genital tract, it can be passed on to the newborn during vaginal delivery leading to either ordinary colonization, or neonatal infections notably umbilical stump sepsis, scalded skin syndrome, arthritis, or bacteraemia/sepsis. These infections are mediated by staphylococcal virulence factors such as (i) Staphylococcal Enterotoxins A, B, C, D, and E encoded by the *sea*, *seb*, *sec*, *sed*, *see* genes, (ii) Exfoliative Toxins A and B encoded by the *eta* and *etb* genes, (iii) Toxic Shock Syndrome Toxin 1 (TSST-1) encoded by the *tst* gene, (iv) Panton-Valentine Leukocidin (PVL) encoded by the *pvl* gene, and (v) Hemolysins alpha and delta encoded by the *hla* and *hld* genes, respectively. We determined the prevalence of *S. aureus* possessing one or more virulence factor genes and of methicillin resistant *Staphylococcus aureus* (MRSA) in this population.

Methods This was a cross-sectional study, which used 85 *S. aureus* isolates from the Chlorohexidine (CHX) clinical trial study in Uganda. The isolates had been obtained by culturing vaginal swabs (VS) from 1472 women in labour, frozen at minus 80°C, then thawed, sub-cultured, and tested for the selected virulence genes *sea*, *seb*, *sec*, *sed*, *see eta*, *etb*, *tst*, *pvl*, *hla* and *hld*, and for the methicillin resistance determining gene (*mecA*). Data were analyzed using SPSS version 20.

Results Of the 85 *S. aureus* isolates 13 (15.3%) were positive for one or more virulence factor genes, as follows: *pvl* 9/85 (10.6%), *hld* 5/85 (5.9%), *sea* 1/85 (1.2%) and *seb* genes 1/85 (1.2%). The other virulence genes (*sec*, *sed*, *see*, *eta*, *etb*, *hla* and *tst)* were not detected in any of the isolates. MRSA was detected in 55.3% (47/85) of the isolates, but only two of these carried the *pvl* virulence gene.

Conclusion This study demonstrated that 15% of the *S. aureus* colonizing the female lower genital tract of mothers in labour in central Uganda carried one or more virulence genes, mostly *pvl*, indicating potential for newborn infection with *S. aureus* acquired in the maternal birth canal. More than half of the isolates were MRSA.

Keywords *Staphylococcus aureus*, Vaginal colonization, Virulence factors, Virulence genes, Female genital tract, MRSA

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Background

Staphylococcus aureus is one of the Gram positive coccal bacteria colonizing the human body as part of the normal flora of the skin, nasal mucosa, the upper respiratory tract and the female lower genital tract [1]. While it can live simply as a normal flora, *S. aureus* is also a known common pathogen of humans where it causes local infections such as cellulitis, boils, surgical site infections, pyomyositis, as well as systemic infections such as acute endocarditis, pyelonephritis, osteomyelitis, septicemia and meningitis [2]. As part of its pathogenesis, *Staphylococcus aureus* is easily transferred between individuals both in the community and health care settings via direct person to person contact [3]. Another mode of transmission is mother to child where babies acquire the organisms from the maternal female lower genital tract during birth.

While *S. aureus* doesn't usually cause infection in the lower female genital tract of pregnant mothers, babies come in direct contact with this organism in the vaginal canal and perineum during birth. The passed on *S. aureus*, may colonize the mouth, the skin, and umbilical stump [4]. This may result into neonatal infections such as Staphylococcal Scalded Skin Syndrome (SSSS), cellulitis, umbilical stump infection (Omphalitis), and arthritis. Among the most severe complications of these *S. aureus* infections are neonatal bacteremia, meningitis and sepsis. Sepsis is a result of vascular damage, extra cellular fluid loss, hypotension, followed by multiple organ failure and death. By itself, neonatal sepsis is the third most common cause of neonatal mortality globally, accounting for 225,000 deaths every year with the highest mortality rates in sub Saharan Africa [5]. A study conducted by Tumuhamye et al. (2020) found that *S. aureus* contributed to 63% of the bacteria isolated from blood cultures of neonates with clinical signs of sepsis admitted to Mulago National Referral Hospital, Kampala, Uganda. [6] Generally, infections caused by *S. aureus* are mediated by different virulence factors encoded by genes located on the chromosome or on mobile genetic elements [7]. The virulence factors and their respective encoding genes include **(i)** Staphylococcal Enterotoxins: SEA, SEB, SEC, SED and SEE encoded by the *sea*, *seb*, *sec*, *sed*, and *see* genes **(ii)** Exfoliative toxins: ETA and ETB encoded by the *eta* and *etb* genes **(iii)** Toxic Shock Syndrome Toxin 1 (TSST-1) encoded by the *tst* gene **(iv)** Panton-Valentine Leukocidin (PVL) encoded by the *pvl* gene and **(v)** Hemolysins alpha and delta encoded by the *hla* and *hld* genes, respectively [8].

Among the neonates, the virulence factors underlying staphylococcal disease include exfoliative toxins which mediates the Staphylococcal scalded skin syndrome (SSSS), and Staphylococcal enterotoxins (SE) A to E, which mediate septic shock [9, 10]. Other virulence factors such as α- hemolysins are implicated in neonatal focal infections such as arthritis and osteomyelitis [8]. Other toxins such as the Panton-Valentinee Leukocidin (PVL) are responsible for the increasing incidence of primary deep-seated folliculitis and necrotizing pneumonia mostly in association with community-acquired methicillin resistant *S. aureus* (CA-MRSA) infections [8, 11].

Despite the clinical signs of *S. aureus* described above, in Uganda, data on the prevalence of virulent *S. aureus* colonizing the female lower genital tract of mothers in labour remains limited. However, a study in the USA found recto-vaginal *S. aureus* colonization of mothers during pregnancy to be 17% (13% for MSSA and 4% for MRSA) but virulence factors were not studied [12]. Another study on relationship between Maternal and Neonatal *Staphylococcus aureus* colonization found that colonization (including MRSA) was extremely common in this cohort of maternal-infant pairs. [15]. Unfortunately, virulence factors were not studied. A study in Uganda in 2020 found that 121 of 1472 women in Labour (8.2%) were colonized by *S. aureus* but neither the prevalence of colonization with virulent *S. aureus* nor the dominant virulence genes was studied [6]. In our study, the aim was to determine the prevalence of *S. aureus* possessing one or more virulence factor genes and also to determine the dominant virulence genes with a focus on the Enterotoxins genes *sea*, *seb*, *sec*, *sed*, and *see* **ii.** Exfoliative toxins genes *eta* and *etb* **iii.** Toxic Shock Syndrome Toxin 1 (TSST-1) genes *tst* **iv.** Panton-Valentine Leukocidin (PVL) genes *pvl* and **v.** Hemolysins alpha and delta genes *hla* and *hld*. Besides possession of virulence factors, *S. aureus* is also of global concern due to its increasing resistance to antimicrobial agents, particularly methicillin resistant *Staphylococcus aureus* (MRSA), which confers resistance to almost all beta lactam agents except ceftaroline and ceftabiprole [13–15]. Thus, a second aim of this study was to determine the burden of *S. aureus* possessing the *mecA* gene which encodes for methicillin resistant *Staphylococcus aureus (MRSA)*.

A study of virulence factors/genes and MRSA has implications for understanding the likelihood of acquisition of virulent and/or resistant *S. aureus* during vaginal delivery with resultant neonatal sepsis. In the developed countries, *Streptococcus agalactaie* is reported to be the commonest cause of early neonatal sepsis, and pregnant mothers are routinely screened for *Streptococcus agalactaie* during the 3rd trimester of pregnancy [16]. Those found to be positive are treated with penicillin to prevent neonatal sepsis [16]. Since *S. aureus* has been found to be the commonest cause of neonatal sepsis in Uganda [17], data from our study might be useful in guiding the development of policies on screening Ugandan pregnant mothers for vaginal colonization with virulent *S. aureus* during the third trimester of pregnancy or during labour.

Materials and methods

Study design, site and settings

We conducted a descriptive cross-sectional study from February to May 2019 at three health centers including Kawaala HC III, Kitebi HC III and Mukono HC IV in urban and peri-urban Kampala, Uganda. The study was nested within the chlorohexidine (CHX) clinical trial, which compared the risk of omphalitis and severe neonatal illness among neonates who underwent umbilical cord cleansing with a single application of 4% CHX at birth versus dry cord care among Ugandan babies born in health facilities [18]. The parent study was funded by the Centre for Intervention Science in Maternal and Child Health (CISMAC), University of Bergen, Norway. In that parent study, 1472 High Vaginal Swabs (HVS) samples were collected from women in labour and cultured to isolate potential bacterial pathogens that colonize the lower female genital tract [19]. From this CHX study, 121 isolates of *S. aureus* were obtained and cryopreserved at -80°c. These were the isolates used for the current study. All laboratory work was performed at MBN Clinical Laboratories (MBN) located at plot 28 Nakasero Road Kampala, Uganda. MBN is a complex of laboratories specialized in Microbiology diagnostics, Molecular diagnostics, DNA Relationship Testing, Immunoassays, Haematology, and Clinical Chemistry Laboratory Diagnostics. The laboratory undergoes external quality assessment/proficiency testing provided by: **i.** Human Quality Assessment Services (HUQAS) Nairobi for Microbiology, Haematology and Clinical Chemistry **ii.** One World Accuracy Canada for SARS-CoV-2 PCR testing, and **iii**. Collaborative Testing Services (CTS), Sterling, VA, USA for DNA testing. MBN is also AABB Accredited. AABB stands for Association for the Advancement of Blood & Biotherapies, *formerly* American Association of Blood Banks based in Bethesda, MD, USA.

Study population

Stored isolates of *S. aureus* collected from women in labour in Kawaala, Kitebi and Mukono health centres under the CHX study were studied.

Sample size determination

The sample size for this study was calculated based on the equation for calculation of sample size for frequency in a population available at 'OpenEpi, v.3, open source calculator-SSPropor' (http://www.openepi.com/Sample-Size/SSPropor.htm) last accessed on 3rd July 2018.

$$\text{Sample size, n} = \frac{[\text{DEFF*Np}\,(\,1-\text{p}\,)]}{[(\,\text{d2/Z21} - \alpha/2 * (\text{N}-1) + \text{p} * (1-\text{p})]}$$

Where,

N = Population size, which in our case is 121 frozen *S. aureus* isolates.

p = hypothesized % frequency of any of the virulence genes in the population (N): 50%+/-5.

d = Confidence limits as % of 100 (absolute +/- %): 5% i.e. 95% confidence level.

DEFF = Design effect (for cluster surveys-DEFF): 1.

By fitting the value into the formulas, the sample size was calculated to be **93**S. *aureus* isolates.

Sampling techniques

Consecutive sampling was used. All 121 isolates that had been phenotypically identified as *S. aureus* under the CHX study were consecutively retrieved from the −80°C freezer, thawed and sub-cultured on 7% sheep blood Agar (Biolabs, Budapest, Hungary). Isolates were re-identified as *S. aureus* based on gram positive coccal morphology, positive catalase test, positive tube coagulase and DNAse tests. Of the 121 frozen isolates, 91 grew upon sub culturing, and 85 of these were re-confirmed *as S. aureus* based on all the four identification criteria set for this particular study.

PCR detection of *S. Aureus* virulence genes

We conducted a multiplex PCR to determine the proportion of *S. aureus* possessing virulence factors and methicillin resistant *Staphylococcus aureus* (MRSA) genes. The PCR testing involved Nucleic acid (DNA) extraction, PCR reagent preparation, DNA amplification, gel electrophoresis and interpretation of results.

Nucleic acid extraction

DNA was extracted using thermo-lysing method locally developed at the laboratory. A 10-μL loop was used to harvest 3–5 pure colonies of *S. aureus* grown overnight on blood agar (Biolabs, Budapest, Hungary). The colonies were emulsified in 500 μl of PCR water in sterile eppendorf tubes and vortexed to wash off media salts from the colonies. The tubes were centrifuged for 10 min at 15,800 RCF (Micromax Centrifuge, model 230, part number 35910889, US). The supernatant was aspirated off and the bacterial cell pellet re-suspended in 200μL of PCR water, and incubated at 100°C for 30 min on a heat block. Tubes with bacterial lysates were cooled for 30 min to room temperature, and then centrifuged for 10 min at 15,800 RCF (Micromax, mode 230, part number 35910889, US). A total of 50μL of the supernatant containing the extracted DNA was transferred into a new eppendorf tube and immediately used for PCR reactions or frozen at minus 80° until used for PCR.

PCR reagent preparation

From the pre-amplification room, PCR reactions were prepared in a total volume of 35 μl consisting of the

following reaction component: 25ul of 2X taq PCR master mix (Qiagen cat# 1067520), 5ul of PCR water and 1 µL (100ng/µl) of each of the forward/reverse primes (Integrated DNA Technologies, Whitehead Scientific, Cape Town, South Africa), whose sequence and amplicon size are shown in the Table 1 [20–23]. The PCR reaction tubes were then transferred to the DNA extraction room and 2 µl of extracted DNA added. The multiplex PCR-testing primers for the various genes was performed as follows:

Set A: *sea, seb, sec, sed and see* genes; **Set B**: *mecA, eta, etb* and *tst* genes; **Set C**: *hla*, and *hld* genes; and **Set D**: 16S r*RNA, pvl, mecA*, and *femA* genes as detailed in Table 1.

Amplification
The PCR tubes were loaded into the Gene Amp PCR system 9700 (Applied Biosystems, Inc. Forster City, CA). The PCR reaction tubes were incubated at 94^{0}C for 5 min

Table 1 Target gene, reagents and primer sequences for PCR sets a, B, and C

Target Gene	PCR reagents and primer sequences (5`- 3`)	Reference	Size of amplified products (bp)	Volume per PCR reaction
Set A genes	**Multiplex PCR Set A reagents and primers**			
	PCR master mix (DNA polymerase, dNTPs, MgCl$_2$**)**	NA	NA	25 µl
sea	*Forward*-GGTTATCAATGTGCGGGTGG	[20]	102	1 µl (100ng)
	Reverse-CGGCACTTTTTTCTCTTCGG			1 µl (100ng)
seb	*Forward*-GTATGGTGGTGTAACTGAGC	[20]	164	1 µl (100ng)
	Reverse-CCAAATAGTGACGAGTTAGG			1 µl (100ng)
sec	*Forward*-AGATGAAGTAGTTGATGTGTATGG	[20]	451	1 µl (100ng)
	Reverse-CACACTTTTAGAATCAACCG			1 µl (100ng)
sed	*Forward*-CCAATAATAGGAGAAAATAAAAG	[20]	278	1 µl (100ng)
	Reverse-ATTGGTATTTTTTTTCGTTC			1 µl (100ng)
see	*Forward*-AGGTTTTTTCACAGGTCATCC	[20]	209	1 µl (100ng)
	Reverse-CTTTTTTTTCTTCGGTCAATC			1 µl (100ng)
	Total Volume of reagents for Set A multiplex PCR	**NA**	**NA**	**35 µL**
Set B genes	**Multiplex PCR Set B reagents and primers**:	[20, 22]		
	PCR master mix (DNA polymerase, dNTPs, MgCl$_2$**)**	NA	NA	25 µl
mecA	*Forward*-ACTGCTATCCACCCTCAAAC	[20]	162	1 µl (100ng)
	Reverse-CTGGTGAAGTTGTAATCTGG			1 µl (100ng)
eta	*Forward*-GCAGGTGTTGATTTAGCATT	[20]	93	1 µl (100ng)
	Reverse-AGATGTCCCTATTTTTGCTG			1 µl (100ng)
etb	*Forward*-ACAAGCAAAAGAATACAGCG	[20]	226	1 µl (100ng)
	Reverse-GTTTTTGGCTGCTTCTCTTG			1 µl (100ng)
tst	*Forward*-ACCCCTGTTCCCTTATCATC	[20]	326	1 µl (100ng)
	Reverse-TTTTCAGTATTTGTAACGCC			1 µl (100ng)
	Total Volume of reagents for Set B multiplex PCR reaction		**NA**	**33 µl**
Set C genes	**Multiplex PCR Set C reagents and primers**:			
	PCR master mix (DNA polymerase, dNTPs, MgCl$_2$**)**	NA	NA	25 µl
hla	*Forward*-CTGATTACTATCCAAGAAATTCGATTG	[21]	209	1 µl (100ng)
	Reverse-CTTTCCAGCCTACTTTTTTATCAGT			1 µl (100ng)
hld	*Forward*-AAGAATTTTTATCTTAATTAAGGAAGGAGTG	[21, 24]	111	1 µl (100ng)
	Reverse-TTAGTGAATTTGTTCACTGTGTCGA			1 µl (100ng)
	Total Volume of reagents for Set C multiplex PCR reaction		NA	**29 µl**
Set D genes	**Multiplex PCR Set D reagents and primers**:			
	PCR master mix (DNA polymerase, dNTPs, MgCl$_2$**)**	NA	NA	25 µl
16 S r*RNA*	*Forward-AACTCTGTTATTAGGGAAGAACA*	[22]	756	1 µl (100ng)
	Reverse-CCACCTTCCTCCGGTTTGTCACC			
pvl	*Forward*-ATCATTAGGTAAAATGTCTGGACATGATCCA	[24]	433	1 µl (100ng)
	Reverse-GCATCAAATGTATTGGATAGCAAAAGC			1 µl (100ng)
mecA	*Forward*-ACTGCTATCCACCCTCAAAC	[20]	162	1 µl (100ng)
	Reverse-CTGGTGAAGTTGTAATCTGG			1 µl (100ng)
femA	*Forward*-AAAAAAGCACATAACAAGCG	[20]	132	1 µl (100ng)
	Reverse-GATAAAGAAGAAACCAGCAG			
	Total Volume of reagents for Set D multiplex PCR reaction		NA	**31 µl**

Abbreviations sea: staphylococcal enterotoxin A, seb: staphylococcal enterotoxin B, sec: staphylococcal enterotoxin C, sed: staphylococcal enterotoxin D, see: staphylococcal enterotoxin E, hla: Alpha hemolysin, hld: Delta hemolysin genes

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followed by 37 cycles of denaturation at 94^0C for 45 s, annealing at 57^0C for 2 min and extension at 72^0C for 1 min as published but with minor modifications [20]. The PCR reactions tubes were finally incubated at 72^0C for 10 min and the PCR products stored at 4^0C until agarose gel electrophoresis.

Agarose gel electrophoresis

A 2% agarose was prepared by weighing 2.0 g of agarose powder (Fisher Scientist, US, Lot #: 200682) in 100 mL of 1x Sodium Borate buffer (SB) catalog number 41920052 (Bioworld, Dublin, OH, USA). The mixture was boiled in microwave oven for 5 min to allow thorough heating and mixing of the powder. The mixture was allowed to cool to 50ºC and then 5 µl Ethidium Bromide (EthBr) catalogue number MFCD00011724 (Sigma-Aldrich, St.

Louis, Missouri, USA) was added. The dissolved agarose solution was then poured into an assembled gel tray with combs attached and allowed to set at room temperature for approximately one hour. Upon setting, the gel was placed into the electrophoretic tank and the combs vertically removed. One microliter of the loading dye catalogue number A3223 (Biomatik, Wilmington, Delaware, USA) was added into each PCR tube with amplicon, mixed well and then 10 µl loaded into the wells. For each electrophoretic run, a 1 kb DNA ladder Lot# 069 (Biomatik, Wilmington, Delaware, USA) was included as molecular weight marker. DNA was electrophoresed at 120 Volts for 30 min. The gel was carefully transferred to a UV Trans-illuminator (Vilber Lournet, France) for visualization. Examples of the gel images for the different genes are shown in Fig. 1.
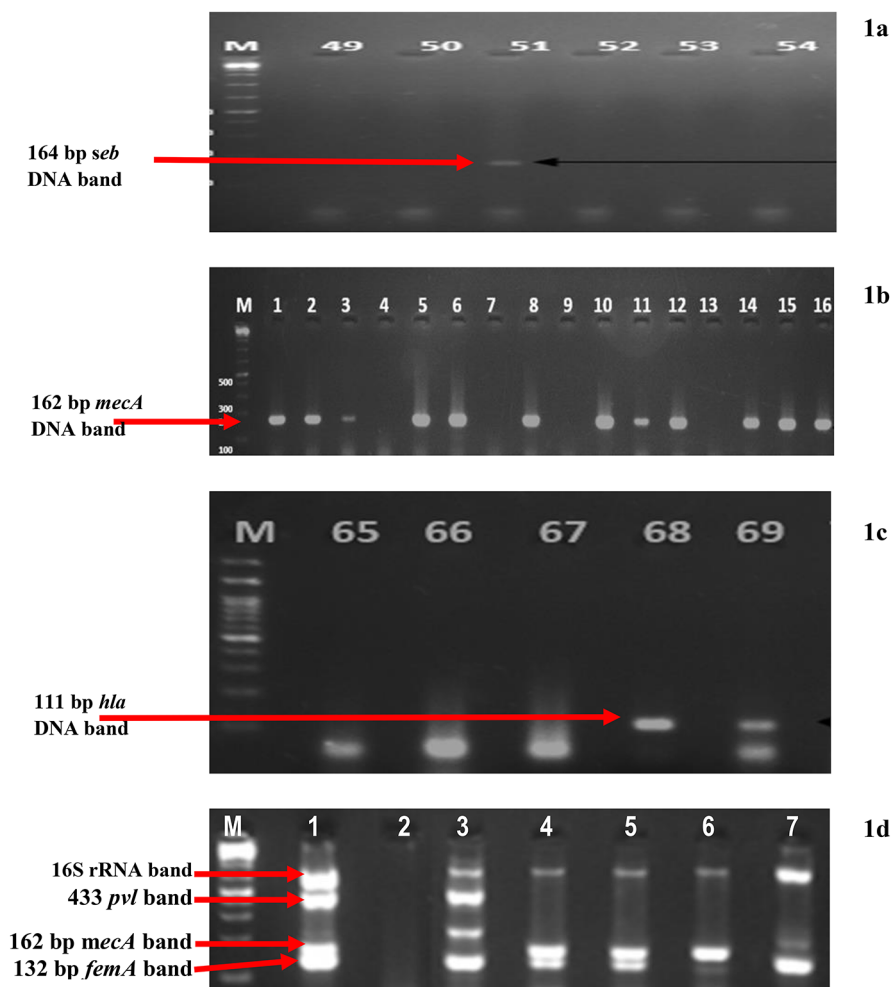
Fig. 1 Gene Electrophoresis Images of various S. aureus genes **(a)** Set A PCR (sea, seb, sec, sed, and see) gel image. Lane M = 1 kb DNA Ladder, Lanes 51 = Sample Positive for seb gene (164 bp). Rest of Lanes = Samples negative for sea, seb, sec, sed, and see). **(b)** Set B PCR (mecA, eta, etb, and tst) gel image. Lane M = 1kb DNA Ladder, Lane 5 = mecA Positive Control, Lane 4 = mecA Negative Control, Lanes 1, 2, 3, 5, 6, 8, 10 to 12, 14 to 16 = Samples positive for mecA gene (162 bp), Rest of the lanes = Samples negative for mecA and other tested genes, eta, etb, & tst. **(c)** Set C PCR (hla and hld) gel image. Lanes M = 1kb DNA Ladder, Lanes 68 and 69 = samples positive for hld gene (111 bp). Rest of the lanes = Samples negative for hld and hla genes.d. Set D PCR (16S rRNA, pvl, mecA, and femA) gel image Lane M = 1kb DNA Ladder. Lane 1 = Pos Control containing 16S rRNA, pvl, mecA, and femA genes (433 bp). Lane 2 = Neg Control (PCR Water) Lanes 4,6,7,9,10,12,13,14,16 = S. aureus pos for femA Lanes 12 = S. aureus pos for pvl gene

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Table 2 Characteristics of studied mothers (data was available for 75 of the 85 mothers)

Characteristic		Frequency (%)
Health centre	**Kitebi, n (%)**	25 (33.33)
	Mukono, n (%)	17 (22.67)
	Kawaala, n (%)	33 (44.00)
Highest level of education of mother	**Tertiary, n (%)**	7 (9.33)
	Secondary, n (%)	43 (57.33)
	Primary, n (%)	24 (31.99)
	None, n (%)	1 (1.33)
Marital status	**Co-habiting, n (%)**	41 (54.67)
	Married, n (%)	13 (17.33)
	Single, n (%)	21 (28)
Husband has any other wife	**Don't know, n (%)**	7 (12.96
	No, n (%)	34 (62.96)
	Yes, n (%)	13 (24.07)
How many times have you been pregnant?	**1, n (%)**	29 (38.67)
	2, n (%)	22 (29.33)
	3, n (%)	10 (13.33)
	4, n (%)	11 (14.67)
	> 4, n (%)	3 (3.99)
What type of toilet do you use?	**Flush toilet, n (%)**	4 (5.33)
	Open pit, n (%)	3 (4.00)
	Pit latrines, n (%)	53 (70.67)
	VIP latrine, n (%)	15 (20.00)
Do you share toilets with any neighbouring households?	**No, n (%)**	15 (20.00)
	Yes, n (%)	60 (80.00

Table 3 *S. aureus* possessing virulence genes (n = 85)

Virulence gene	No. of *S. aureus* isolates (%)
None (No *sec, sed, see, eta, etb, hla, tst genes*)	72 (84.7)
Pvl	9 (10.6)
hld	5 (5.9)
Sea	1 (1.2)
Seb	1 (1.2)

participants were either co-habiting or married. The percentage of mothers who were in labor for the first, second or third time all together contributed to over 81% as referred to Table 2.

Prevalence of *Staphylococcus aureus* possessing one or more virulence factor genes

Out of the 85 studied isolates, 13 (15.3%) were positive for one or more virulence factor genes. The detected virulence genes were *pvl*, *hld*, *sea* and *seb* in 9 (10.6%), 5 (5.9%), 1 (1.2%) and 1 (1.2%), respectively as shown in Table 3. Three isolates had two virulence genes each i.e. two isolates with *pvl* & *hld*, and one isolate with *seb* & *hld*. The other studied virulence genes *i.e. sec*, *sed*, *see*, *eta*, *etb*, *hla*, and *tst* were not detected in any of the isolates.

Looking at the individual 16 virulence genes, *pvl* and *hld* were the most frequently possessed by the *S. aureus* in 9 (56.3%) and 5 (31.3%) of the isolates. The other genes *sea and seb* were detected in only one (6.2%) isolate each.

Prevalence of *S. aureus* carrying the *mecA* gene

Of the 85 confirmed *S. aureus* isolates, 47 (55.3) % possessed the *mecA* gene. Of the 47 *mecA* positive isolates, only two were also positive for the *pvl* gene, the other 45 remained negative for any virulence gene.

Quality control testing

For the phenotypic re-identification of *S. aureus*, a known *S. aureus* strain (ATCC 25923,) was used as positive control and known *S. epidermidis (ATCC 14990,)* used as a negative control (Manassas, 10801 University Blvd, United States). Each PCR batch had positive and negative controls for some of the genes under study as well as PCR water to check for reagent contamination possibilities. These positive controls were laboratory cocktails containing some of the genes under study as well and *S. aureus* 16s rRNA genes.

Data analysis

All study data was entered in Ms Excel 2013 and analyzed using SPSS v.20. (https://www.ibm.com/support/pages/downloading-ibm-spss-statistics-20)

Results

Population Characteristics

We studied 85 *S. aureus* isolates coming from 85 mothers. The median age of the mothers was 23 with the range of 20 to 27 years. Over 77% of the studied participants came from Kawala and Kitebi Health centers. Over 89% of the participants had achieved either primary or secondary school education. Slightly over 72% of the

Discussion

Our study explored the prevalence of *S. aureus* isolated from the female lower genital tract (FGT) of mothers in labour possessing selected virulence factor genes in Uganda, and determined which virulence genes dominate. To our knowledge, this is the first study that looked for virulence genes in *S. aureus* isolates from the lower FGT of mothers in labour in the study settings. The finding of 13 isolates (over 15% of the isolates) positive for one or more virulence factor genes and a total of 16 virulence factor genes is important. The dominance of the *pvl* gene in the studied *S. aureus* isolates is worrying because this particular virulence gene encodes for a toxin called Penton-Valentine Leukocidin (PVL). The PVL toxin causes formation of trans-membrane pores in leukocytes, causing them to degranulate and extra cellular pouring of leukocyte contents into the extra cellular space causing

extensive enzymatic soft tissue damage, generalized deep folliculitis, marked generalized inflammation, fatal necrotizing pneumonia and often death [24].

Our findings of *pvl* gene in 10.6% participants were similar to those in a study conducted in China by Yuh *et al. (2008)*, where they found a prevalence of 11.9% of *Staphylococcus aureus* carrying Panton–Valentinee leukocidin genes among isolates from hospitalized patients in China. However, in their study, samples other than vaginal specimens were examined [23]. Also, *pvl* prevalence in our study was similar to the findings from Nigeria which found a prevalence of *pvl* genes to be 10.7% [25]. The *pvl* gene prevalence in our study was lower compared to the one detected by Schaumburg et al (2014) in their study entitled transmission of *Staphylococcus aureus* between mothers and infants in an African setting; they found a prevalence of *pvl* positive isolates to be 56.7% [26]. Another study by Bastidas et al (2019) on antibiotic susceptibility profile and prevalence of *mecA* and *pvl* genes in *Staphylococcus aureus* isolated from nasal and pharyngeal samples, found a lower prevalence of 3.2% of *pvl* genes compared to our study [27].

In our study, the prevalence of *hld* gene, which encodes for the Delta-hemolysin was 5.9%. A study conducted by Mohamed et al.(2018) on identification of hemolysin genes and their association with antimicrobial resistance pattern among clinical isolates of *Staphylococcus aureus* found a higher prevalence of 11.59%) [28]. The Delta-hemolysin is a cytolytic and cytotoxic toxin associated with erythrocytes lysis, severe skin infections, pneumonia, and sepsis. It is fortunate that our study found a low prevalence of these genes and this may imply that newborns from those mothers are probably less likely to acquire infections mediated by these virulence factors.

Among the 5 staphylococcal enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) we detected only *sea* and *seb* in very low prevalence of only 1.2% for each. This is in contrast to findings in a study by Sultan et al (2019) on clinical *S. aureus* isolates cultured from wound swabs, blood, endocarditis, bone marrow, urine, abscesses, ear swab, throat swab and sputum [29]. In their study, they found *sea* gene prevalence of 48.31%, and *seb* gene to be 44.94% far higher than in our study, probably because they studied clinical isolates from active infection lesions. They also detected *sec*, *sed*, *see*, *tst*, *eta*, and *etb* in prevalence of 6.74%, 3.37%, 16.85%, 86.51%, 5.61% and 2.24%, respectively unlike in our study, where none of the latter 6 genes was detected. The fact that their study used clinical isolates from active infections might explain why the prevalence was higher. The *seb* gene codes for the Staphylococcal enterotoxin B (SEB) which is an exotoxin and a superantigen capable of immunomodulation of pro-inflammatory mediators. It is also capable of causing food poisoning [30]. Based on our findings, those *S.*

aureus isolates from the lower genital tract of mothers in Labour in Uganda appear to be less virulent, and neonates born of those mothers carrying *S. aureus* are less likely to get any of those complications.

Methicillin resistant *S. aureus* (MRSA) encoded by the *mecA* gene is one of the most dangerous strains of *S. aureus* today, as it resists almost all beta lactams except ceftaroline and ceftobiprole [13]. MRSAs are also resistant to a wide range of other classes of antimicrobial agents particularly if they are hospital acquired [31]. Our finding of a high *mecA* gene prevalence of 55% is very worrying since beta-lactam drugs are the most widely used agents in empirical treatment of neonatal infections in Uganda and in many resource-limited settings, yet they would not work when it comes to MRSA. Our study appears to be the first in determining MRSA prevalence among *S. aureus* isolates colonizing the lower FGT of mothers in labour in the study settings. The other studies on MRSA prevalence were on different samples but found prevalence values similar to our findings. For example, a study in Kenya by Wangai et al. (2019) on MRSA in East Africa reported an overall MRSA prevalence of 53.4% though the majority of isolates in that study were from skin and soft tissue infections [32]. Another study by Masaisa et al. (2018) on antibiotic patterns and molecular characterization on MRSA in clinical settings in Rwanda in different clinical samples of patients attending a referral hospital in Kigali found the overall prevalence of MRSA to be (33.3%) [33]. Kateete et al. (2011) in their study at Mulago national referral hospital in Kampala, Uganda on prevalence of Methicillin resistant *Staphylococcus aureus* in surgical units found a lower prevalence of 46% [34]. Another study done by wekesa et al. (2018) done on bacterial species and antibacterial resistance among post caesarean section surgical site infections at Mulago hospital Kampala, Uganda demonstrated that 88.2% were Methicillin resistant *S. aureus* (MRSA). Another study by Tumuhamye et al. (2020), on neonatal sepsis at Mulago national referral hospital in Uganda found *S. aureus* to be the most predominant bacteria isolated from those neonates and 5.3% of those were MRSA [6, 35].

Our findings have implications in that those mothers colonized by MRSA might transmit them to the neonates during birth. Once newborns acquire such virulent and drug resistant strains it is a challenge because it is very difficult and expensive to treat due to their resistance to many classes of antibiotics such as the readily available beta lactams. Fortunately, we found no virulence genes in all except two MRSA isolates.

We attempted to look for but didn't find any association between *mecA* and *pvl* gene since of the 47 *mecA* positive isolates, only two were also positive for the *pvl* genes, meaning that whereas MRSA strains of *S. aureus*

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dominated in the lower FGT of mothers in labour, most of these were most likely avirulent. Our findings are similar to those in other studies, but which studied different clinical situations. Karmaka et al (2018) found the prevalence of *pvl* in MRSA to be low (9%) among community acquired *Staphylococcus aureus* [36]. Motamedi et al (2015) who studied association of Panton-Valentinee leukocidin and *mecA* genes *Staphylococcus aureus* isolates from patients referred to educational hospitals in Ahvaz, Iran found none of the *mecA* positive isolates with the *pvl* gene [37]. Also, another study in Nigeria on association of virulence genes with *mecA* gene in *Staphylococcus aureus* isolates from tertiary hospitals by Alli et al. (2015) found the prevalence of the *pvl* gene in only 9.1% of MRSA compared with 53.3% among the methicillin susceptible *Staphylococcus aureus* (MSSA) [38].

Limitations

We retrieved 121 frozen isolates but only 85 isolates were able to grow on subculture and confirmed as *S. aureus*. Our study is therefore limited by the small sample size.

Conclusion

We have found that 15% of the *S. aureus* colonizing the female lower genital tract of mothers in labour in Uganda carried one or more virulence genes, majorly *pvl* and *hld* genes. This implies that the potential for newborn acquisition and possible infection with virulent *S. aureus* stands at approximately one in every 6 newborns. MRSA was found in more than half of the isolates but these isolates were mostly avirulent.

Declarations

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