Nasopharyngeal carriage, spa types and drug susceptibility profiles of Staphylococcus aureus from healthy children under 5 years in eastern Uganda

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Abstract

Background: Staphylococcus aureus carriage is a known risk factor for staphylococcal disease. However, the carriage rates vary by country, demographic group and profession. This study aimed to determine the S. aureus carriage rate in children in Eastern Uganda, and track Staphylococcus strains that can cause infection in Uganda. Methods: Nasopharyngeal samples from 742 healthy children under 5 years residing in the Iganga/Mayuge Health & Demographic Surveillance Site in Eastern Uganda were processed for isolation of S. aureus. Antibiotic resistance based on minimum inhibitory concentrations (MICs) was determined by the BD Phoenix™ automated identification & susceptibility testing system. Genotyping was performed by spa typing. Results: The processed samples yielded 144 S. aureus isolates (one per child) therefore, the S. aureus carriage rate in children was 19.4% (144/742). Thirty one percent (45/144) of the isolates were methicillin resistant (MRSA) yielding a carriage rate of 6.1% (45/742). All MRSA isolates were susceptible to vancomycin, linezolid and clindamycin however, compared to methicillin susceptible S. aureus (MSSA) (68.8%, 99/144), MRSA isolates were more resistant to non-beta-lactam antimicrobials -tetracycline (91.1%, 41/45), trimethoprim/sulfamethoxazole (73.3%, 33/45), erythromycin (75.6%, 34/45), chloramphenicol (60%, 27/45), gentamicin (55.6%, 25/45) and ciprofloxacin (35.6%, 16/45). Furthermore, one MRSA isolate was mupirocin resistant and 42 (93.3%, 42/45) were multidrug resistant (MDR); three (3%, 3/99) MSSA isolates were mupirocin and clindamycin resistant, while 61 (61.6%, 61/99) were MDR. All MSSA/MRSA isolates were susceptible to rifampicin, vancomycin and linezolid but only three MSSA were pan-susceptible to antibiotics. Seven spa types were detected in MRSA, of which t037 & t064 were predominant and associated with SCCmec types I & IV, respectively. Fourteen spa types were detected in MSSA, of which t645 & t4353 were predominant. Conclusions: S.
*Staphylococcus aureus* (MSSA/MRSA) carriage rate in children in Eastern Uganda is high and comparable to rates for hospitalized patients in Kampala city. The detection of mupirocin resistance is worrying as it could rapidly increase if mupirocin is administered in a low-income setting. *S. aureus* strains of spa types t064, t037 (MRSA) and t645, t4353 (MSSA) are prevalent and could be responsible for majority of staphylococcal infections in Uganda.

**Background**

A causal relationship between *Staphylococcus aureus* carriage and infection is well documented [1, 2]. The anterior nares and nasopharynx are the most important sites for *S. aureus* colonization in humans [3]. Generally, the prevalence of *S. aureus* nasal carriage ranges from 20% to 30% however, it varies by country, profession and demographic group [2, 4]. Notably, these estimates are from developed countries and their applicability to African settings where infection control practices and surveillance for antimicrobial resistance are inadequate/nonexistent [5], is debatable. Furthermore, information on the prevalence, population structure and molecular epidemiology of *S. aureus* carriage in healthy African individuals is scarce [6]. Although the prevalence and correlates of *S. aureus* nasal carriage in hospitalized adult patients and health workers in Uganda has been documented [7-9], carriage rates in communities especially in children who are more vulnerable to staphylococcal infections, is not known. Generally *S. aureus* studies in Africa have focused on clinical and/or nosocomial isolates, which limits our understanding of the *S. aureus* population structure on the continent [1].

The *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* nasopharyngeal carriage rates among healthy Ugandan children was found to be high at 58.6% (89/152), 15% (23/152) and 11% (16/152), respectively [10]. These bacteria alongside *S. aureus*, are the main causes of pneumonia [10], the leading cause of death in children under five years [11]. In this follow-up study, we aimed to determine the *S.*
*aureus* carriage rate in healthy children under 5 years of age residing at the Iganga/Mayuge Health & Demographic Surveillance Site (IMHDSS) in rural Eastern Uganda, and track *Staphylococcus* strains, both methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA), that can cause infection in Uganda. Due to frequent interaction between health care workers from Mulago Hospital in Kampala city and community members at the IMHDSS, we hypothesized that *S. aureus* isolates from the two settings i.e. Mulago Hospital and IMHDSS will generally be of the same genetic background.

**Methods**

**Study setting and identification of *S. aureus***

This cross-sectional study was nested in a previous study that investigated the nasopharyngeal carriage of pneumococci in healthy children less than 5 years of age at the IMHDSS [10], located in Iganga and Mayuge districts in rural Eastern Uganda, between February and October 2011. Following specimen processing for isolation of *Strep. pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis* during the previous study [10], nasopharyngeal samples (swabs) from 742 children were sub-cultured again for isolation of *S. aureus*, as highlighted below.

**Figure 1** highlights the procedure we followed to isolate and identify *S. aureus*. Briefly, presumptive *Staphylococcus* colonies were re-streaked on blood agar plates or on tryptic soy agar plates (for samples with no growth on blood agar) and incubated at 37 °C for 24 hours in a CO₂ incubator. To identify *S. aureus*, we subjected Gram-positive and catalase-positive isolates to three different methods commonly used to identify *S. aureus*: (i) tube coagulase test, (ii) growth on DNase agar, and (iii) growth on Mannitol salt agar [12]. Isolates that tested positive on all the three methods were considered to be *S. aureus*;
otherwise isolates that tested negative with one or two of the methods were subjected to PCR-detection of the *nuc* gene as previously described [12]; isolates were considered to be *S. aureus* if a 270 base pair fragment was identified on agarose gel electrophoresis. Otherwise *nuc* gene PCR-negative isolates were further evaluated with the BD Phoenix 100 ID/AST expert system for identification as previously described [13-15]. Isolates that tested negative with all the confirmatory tests for identification of *S. aureus* (i.e. tube coagulase test, *nuc* gene PCR & BD Phoenix 100 ID/AST) were excluded from further analysis.

**Drug susceptibility testing**

Except mupirocin susceptibility that was determined by the BD Phoenix 100 ID/AST expert system, drug susceptibility testing was determined by the disc diffusion method according to Clinical and Laboratory Standards Institute, (CLSI, 2011) http://ljzx.cqrmhospital.com/upfiles/201601/20160112155335884.pdf. Briefly, colonies of pure bacterial isolates were suspended in sterile normal saline to a turbidity of McFarland standard 0.5 and uniformly spread on Muller Hinton agar (MHA) plates (Biolabs®, Hungary) with the following antibiotic disks (Biolabs®, Hungary): penicillin G (10 units), cefoxitin (30 µg), clindamycin (2 µg), erythromycin (15 µg), vancomycin (30 µg), tetracycline (30 µg), linezolid (30 µg), trimethoprim/sulphamethoxazole (1.25/23.5 µg), chloramphenicol (5 µg), ciprofloxacin (5 µg), and gentamicin (10 µg). For disc diffusion, plates were incubated at 37 °C for 24 hours. Inhibition zones were measured in millimeters and interpreted as susceptible (S), intermediate (I) or resistant (R). *S. aureus* isolates with inhibition zone diameter of ≤19 mm on 30 µg cefoxitin disk were considered to be MRSA. Twelve cefoxitin susceptible isolates were *mecA* positive but further investigation with the minimum inhibitory concentration (MIC) method found that all the 12 isolates were MRSA by cefoxitin MICs according to CLSI. These isolates were confirmed.
to be MRSA by the BD Phoenix 100 ID/AST expert system.

**Genotyping and other molecular procedures**

To determine the lineages, spa typing was performed as described previously [16]. Briefly, PCR products were purified with QIAquick PCR purification kit (Qiagen) and sequenced at MBN Laboratories (Kampala, Uganda) or ACGT Inc. (Wheeling, IL, USA) using forward and reverse primers used in PCRs. To obtain spa types, spa sequences were submitted to an online spaTyper server (http://spatyper.fortinbras.us/) and confirmed by cross-checking the repeats with the Ridom Spa Server (http://spaserver2.ridom.de/spatypes.shtml). Furthermore, pulse field gel electrophoresis (PFGE) analysis was performed for selected isolates according to Asiimwe et al, 2017 [17]. Briefly, isolates were subjected to Smal digestion using the CHEF Genomic DNA Plug kit (Bio-Rad) according to a standardized procedure [17], and PFGE run using a CHEF DRIII system (Bio-Rad). The InfoQuest FP (v5) software (Bio-Rad Laboratories) was used to analyze the PFGE profiles and interpreted according to Tenover et al. [18]. Cluster analysis was achieved using Dice similarity coefficients and the unweighted pair group method with averages (UPGMA) at 1.5% optimization and 1.5% position tolerance. Isolates displaying ≥95% similarity (≈3 band difference) were assigned the same profile [18]. To detect the PVL-encoding genes (*lukS-PV* & *lukF-PV*), isolates were screened by PCR as previously described [8, 9]. Reference strains ATCC-43300 -mecA+, PVL- (MRSA) and ATCC-29213 -mecA-, PVL- (MSSA) & ATCC-25923 -mecA-, PVL+ (MSSA) were used as positive or negative controls. Apart from spa typing in which all the PCR products were sequenced, DNA sequencing of amplified segments of *mecA* and PVL genes was performed for selected isolates and confirmed by BLAST-searching at NCBI https://blast.ncbi.nlm.nih.gov/Blast.cgi Additionally, we also compared spa types for *S. aureus* at the IMHDSS in Eastern Uganda to previously described spa types for *S. aureus* isolates from Mulago National Referral Hospital in
Kampala [9], as well as pastoral communities in rural western Uganda [17, 19].

Results

**Demographic characteristics**

The characteristics of children sampled were described previously [10, 20]. Briefly, about half of the children were less than 2 years old and 50% were female with a mean age of 30 months. Majority (82%) of the children lived in rural areas and their primary source of water was spring water (74%) and well water (26%); only 1% of the children had access to piped water. The median number of children per household was 2 while the median number of household members per bedroom (as a measure of crowding) was 4. Based on reports of mothers 90% of the children had been sick in the previous 2 weeks and the most common symptoms were fever, running nose and cough. Of the children who had been sick around 30% were reported to have been given antibiotics, mostly cotrimoxazole and ampicillin.

**MSSA and MRSA carriage rates**

The processed samples yielded 600 Gram positive and catalase positive isolates (one isolate per sample/child), of which 144 were confirmed to be *S. aureus* (one isolate per sample/child), Figure 1 and Tables S1 & S2. Thus, the nasopharyngeal carriage rate of *S. aureus* in the children was 19.4% (144/742). Furthermore, 45 (31.3%, 45/144) of the isolates were confirmed to be MRSA yielding a carriage rate of 6.1% (45/742) in the children.

**Spa Types, resistance patterns and antibiotypes**

All MRSA isolates were susceptible to vancomycin, linezolid and generally clindamycin though two exhibited intermediate resistance to this drug, Tables S1 & S2. Compared to MSSA, MRSA isolates were more resistant to non-β-lactam antimicrobial agents i.e. tetracycline (91.1%, 41/45), SXT (73.3%, 33/45), erythromycin (75.6%, 34/99),
chloramphenicol (60%, 19/99), gentamicin (55.6%, 25/45) and ciprofloxacin (35.6%,
16/45). One MRSA isolate (K2283) exhibited high-level mupirocin resistance (HLMup\textsuperscript{r})
while 42 (93.3%, 42/45) were multidrug resistant (MDR – resistance to three or more
classes of antimicrobials) including the mupirocin resistant isolate, \textit{Tables S1 \& S2}. Again, compared to MSSA, slightly more MRSA isolates (13.2%, 19/45) carried \textit{PVL} genes
but the difference was not statistically significant (\textit{P}=0.4562). All PVL-positive MRSA
isolates were MDR and generally the presence of \textit{PVL} genes was associated with the MDR
phenotype (\textit{P}=0.0332). Seven \textit{spa} types (t064, t4353, t002, t037, t355, t3092 and t12939)
were detected among MRSA isolates, of which t064 (20%, 9/45) and t037 (15.6%, 7/45)
were predominant, \textit{Tables S1 \& S2}. \textit{Spa} types t037 and t064 were significantly
associated with MRSA and \textit{SCCmec} types I \& IV respectively, with t037 exclusively
occurring in MRSA isolates, \textit{Table 1}. On PFGE analysis isolates of \textit{spa} type t064 clustered
together, \textit{Figure S1}.

\textbf{Table 1: Distribution of \textit{spa} types in \textit{S. aureus} from children in IMHDSS, eastern Uganda}
| Spa type | MDR (%) | MSSA | MRSA | Total |
|----------|---------|------|------|-------|
|          | Yes     | No   | Frequency | RF | Frequency | RF | Frequency |
| t064     | 10 (66.7) | 05 (33.3) | 06 | 6.1 | 09 | 20 | 15 |
| t645     | 06 (55.4) | 05 (45.5) | 11 | 11.1 | 0 | 0 | 11 |
| t4353    | 06 (60) | 04 (40) | 09 | 9.1 | 01 | 2.2 | 10 |
| t002     | 06 (85.7) | 01 (14.3) | 06 | 6.1 | 01 | 2.2 | 07 |
| t037     | 06 (85.7) | 01 (14.3) | 0 | 0 | 07 | 15.6 | 07 |
| t078     | - | - | 02 | 2 | 0 | 0 | 02 |
| t355     | - | - | 01 | 1 | 01 | 2.2 | 02 |
| t3092    | - | - | 01 | 1 | 01 | 2.2 | 02 |
| t12939   | - | - | 0 | 0 | 01 | 2.2 | 01 |
| t3662    | - | - | 01 | 1 | 0 | 0 | 01 |
| t318     | - | - | 01 | 1 | 0 | 0 | 01 |
| t1456    | - | - | 01 | 1 | 0 | 0 | 01 |
| t10394   | - | - | 01 | 1 | 0 | 0 | 01 |
| t1476    | - | - | 01 | 1 | 0 | 0 | 01 |
| t2168    | - | - | 01 | 1 | 0 | 0 | 01 |
| t213     | - | - | 01 | 1 | 0 | 0 | 01 |
| Unknown  | - | - | 04 | - | 0 | 0 | 04 |
| NT       | - | - | 17 | - | 04 | - | 21 |
| ND       | - | - | 35 | - | 20 | - | 55 |
| Total    | - | - | 99 | - | 45 | - | 144 |

The predominant spa types are depicted in bold font. RF denotes Relative Frequency (%)

Ninety nine (68.8%, 99/144) isolates were MSSA as they were cefoxitin susceptible and mecA negative, Tables S1 & S2. Nevertheless, MSSA isolates were highly resistant to penicillin (78.8%, 78/99), tetracycline (79.8%, 79/99), SXT (27.3%, 27/99), erythromycin (24.2%, 24/99), gentamicin (25.3%, 25/99) and chloramphenicol (19.2%, 19/99) but compared to MRSA, they were not as resistant to ciprofloxacin (2%, 2/99). Three (3%, 3/99) MSSA isolates (K277-1, K251 & K1064) exhibited high-level mupirocin resistance and were also clindamycin resistant, Tables S1 & S2. A total of 61 (61.6%, 61/99) MSSA were MDR including the three mupirocin resistant isolates, Tables S1 & S2. Generally all isolates, MSSA and MRSA, were susceptible to rifampicin, vancomycin and linezolid but only three (R16, R180 & R716) were pan-susceptible to antibiotics. Fourteen spa types were detected among MSSA, of which t645 (11.1%, 11/99), t4353 (9.1%, 9/99), t064
and t002 (6.1%, 6/99) were predominant, Tables S1 & S2. Spa type t645 exclusively occurred in MSSA, Table 1.

Overall, a total of 38 and 28 antibiotypes were detected among MSSA and MRSA isolates, respectively. Table 2 summarizes the antibiotypes and their relationship with spa types and SCCmec types. The most prevalent antibiotypes in MSSA and MRSA had the resistance patterns PEN-TET (17.2%) and FOX-PEN-TET-SXT-ERY-CHL-GEN (15.6%) respectively, Table 2.

Table 2: Antibiotypes among MSSA & MRSA and their relationship with Spa types

| Antibiotype | Resistance profile | # isolates showing this pattern (%) | Major Spa types (f) |
|-------------|--------------------|-------------------------------------|---------------------|
| **MSSA**    |                    |                                     |                     |
| S1          | PEN-TET            | 17 (17.2)                           | t064 (4), t4353 (3) |
| S2          | PEN-TET-ERY       | 11 (11.1)                           | t002 (3)            |
| S3          | PEN-TET-GEN       | 9 (9.1)                             | t318 (1), t213 (1), |
| S4          | PEN                | 6 (6.1)                             | t002 (1), t645 (1), |
| S5          | TET                | 6 (6.1)                             | t4353 (1)           |
| S6          | PEN-TET-CHL-GEN   | 4 (4.4)                             | t645 (1)            |
| S7          | PEN-TET-SXT       | 4 (4.4)                             | t002 (1), t144 (1)  |
| S8          | PEN-TET-SXT-CHL   | 3 (3)                               |                     |
| S9          | PEN-SXT-ERY-CLI-MUP | 3 (3)                        |                     |
| S10         | PEN-TET-CHL       | 2 (2)                               | t645 (1), t4353 (1) |
| S11         | TET-SXT-CHL       | 2 (2)                               | t3662 (1), t10:     |
| S12         | PEN-TET-ERY-CHL   | 2 (2)                               | t064 (1), t304 (1)  |
| S13         | PEN-SXT           | 2 (2)                               | t4353 (1)           |
| S14         | PEN-TET-SXT-ERY   | 2 (2)                               |                     |
| S38         | - (Pan-susceptible) | 3 (3)                        | t064 (1)           |
| **MRSA**    |                    |                                     |                     |
| R1          | FOX-PEN-TET-SXT-ERY-CHL-GEN | 7 (15.6) | - (4), t064 |
| R2          | FOX-PEN-TET-SXT-ERY-CHL-GEN-CIP | 4 (9)                             | -                   |
| R3          | FOX-PEN-TET-SXT-ERY-CHL-GEN | 4 (9)                             | -                   |
| R4          | FOX-PEN-TET-SXT-ERY | 3 (6.7)                             | t002 (1), t064 (1)  |
| R5          | FOX-PEN-TET-SXT-ERY-CIP | 3 (6.7)                        | t064 (1)           |
| R6          | FOX-PEN-TET-SXT-ERY-GEN | 2 (4.4)                        | t064 (1)           |

Shown are antibiotypes depicted by two or more isolates; antibiotypes depicted by only one isolate are shown in Table S1. FOX, cefoxitin; PEN, penicillin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole or co-trimoxazole; ERY, erythromycin, CHL, chloramphenicol; GEN, gentamycin; CIP, ciprofloxacin; CLI, clindamycin; RIF, rifampicin; MUP, Mupirocin High level; VAN, vancomycin; LZD, linezolid; MSSA, Methicillin susceptible *S. aureus*; MRSA, Methicillin resistant *S. aureus*. 
When we compared the genotypes of *S. aureus* isolates at the IMHDSS to genotypes of previously characterized isolates in Uganda, we found that the *spa* types we detected at IMHDSS were previously reported for *S. aureus* isolates from Mulago Hospital in Kampala, but they were slightly different from *spa* types for isolates from rural Western Uganda,

**Figure S2.** Thus, the predominant lineages in each of the three settings were t064, t645, t4353, t002 & t037 (IMHDSS); t645, t4353, t064, t084, t355, t3772 & t4609 (Mulago Hospital); and t318, t064, t645, t186, t11656, t127, t786 & t2771 (rural Western Uganda), **Figure S2.** Overall, 40 *spa* types account for clinical and colonizing MSSA/MRSA clones circulating in Uganda, of which t645, t064, t4353, t002, t318, t037, t355, t084, t3772, t127 and t186 are predominant, **Figure 2 & Table S3.** Spa type t037 was detected only at Mulago Hospital & IMHDS but not in Western Uganda; again, it exclusively occurred in MRSA. *Spa* types t645 & t4353 occurred in all the three sites and they were significantly associated with MSSA, **Table S3 & Figure 2.** When we analyzed isolates from the three sites together, *spa* types t4353, t002 & t355 were neither associated with MRSA nor MSSA; interestingly, *spa* type t064 that was significantly associated with MRSA at IMHDSS was not associated with MRSA, **Table S3.**

**Discussion**

*Staphylococcus aureus* infection in Uganda was first reported at Mulago National Referral Hospital in 1958 [21]. Since then, *S. aureus* has remained a leading cause of infection in Uganda and beyond [22-31], and it is second only to the pneumococcus among the frequent causes of pneumonia in children in Africa [1, 10, 11, 32]. Nasal/nasopharyngeal carriage of bacteria including *S. aureus* is a main risk factor for hospital- and community-acquired infections, as carriage provides a reservoir from which bacteria spread when the immune system defenses is weakened. In this study, the *S. aureus* nasopharyngeal carriage rate (19.4%) in healthy children in Eastern Uganda was found to be high but it
falls within the reported estimates for children i.e. 18.12%-38.5% [4]. The MRSA carriage rate (6.1%) was also high and comparable to estimates for adult patients at Mulago Hospital in Kampala city [8, 9]. The MRSA carriage rate was higher than the reported rates for children in developed countries (i.e. 0.8%-1.5%) [4, 33] and developing countries (e.g. 2.3%, 14/622 for urban/rural school children in Gondar, Ethiopia [34]; 0.6%, 85/950 for adult inpatients in a Kenyan Government hospital [35]). Several factors could be responsible for the high MRSA carriage rate in Ugandan children e.g. previous exposure to antibiotics as one third of the children had been given ampicillin and cotrimoxazole.

Crowding is another factor that could have contributed to the observed high carriage rates in children as the median number of household members per bedroom at the IMHDSS was four; large households of ≥5 members were reported to be positively associated with high S. aureus nasal carriage rates [1].

In this study, MSSA isolates were highly resistant to penicillin and tetracycline (78-79.8%) while resistance to SXT, erythromycin, chloramphenicol, gentamicin, clindamycin and ciprofloxacin was generally low. These findings are consistent with reports that penicillin resistance rates for S. aureus have remained in this range or higher since the late 1970s [36]. In fact, penicillin resistant S. aureus in Uganda was first reported at Mulago Hospital in Kampala in 1958 [21], at relatively lower prevalence (i.e. 28%, 27/98) compared to current rates (91%). Furthermore, MRSA isolates in this study were MDR and this is consistent with various reports of MRSA strains being MDR [32, 37]. The reported coexistence between hospital-acquired MRSA and community-acquired MRSA strains in Uganda [38] and other countries in Africa [39] likely contributes to the increasingly high MDR-MRSA rates reported from Africa. Clindamycin, rifampicin, vancomycin and linezolid were the most effective antibiotics in this study, in contrast to findings from Ethiopia where gentamycin and ciprofloxacin were the most effective among isolates from primary
school children [34].

Mupirocin is a newer drug used as a topical antibiotic to treat impetigo caused by *S. aureus* and *S. pyogenes*. It is also commonly used for nasal decolonization of MSSA and MRSA in patients and health care workers in the developed countries [5, 32, 36]. We have reported a high rate (4%) of mupirocin resistance (high-level) and this is consistent with its reported rates in Africa (5%-50%) [40]. The detection of mupirocin resistance in Uganda is worrying as MRSA decolonization is generally not a common practice in Africa. As mupirocin use in decolonization regimens leads to rapid emergence of resistance [32], its introduction in African settings for eradication of *Staphylococcus* colonization will likely spike an increase in resistance [40].

Overall, *S. aureus* strains of spa types t645, t064, t4353, t002, t318, t037, t355, t084, t3772 & t127 are prevalent in Uganda and generally in Africa [35, 41, 42]. Consistent with this, a systematic review of the global distribution of spa types revealed that t064 and t037 were the most prevalent spa types in Africa with t037 being exclusively associated with MRSA strains [43]. The exception is t4353, which appears to be common in Uganda [19] but rare in other African countries. Thus, *S. aureus* infections in Uganda are likely to be associated with strains of spa types t064 & t037 (MRSA) and/or t645, t4353, t002 & t318 (MSSA/MRSA). Spa types t037 and t064 are frequently associated with MLST sequence types ST-30 & ST-8, respectively, while t645 is associated ST-121 [44]. It is interesting to note that attempts to type staphylococci in Uganda date 1958 when phage typing was used to characterize *S. aureus* isolates at Mulago Hospital [21]. However, the typing of MRSA is relatively recent in Uganda as it was first reported in 1992 [45] (by multi-locus enzyme electrophoresis).

There were certain limitations in this study. First, few studies have performed spa typing of *S. aureus* isolates in Uganda hence, the reported frequencies and associations should
be taken with caution. Second, we used a cross-sectional design with a single sampling point to classify children as carriers implying that some of the children we classified as non-carriers might have been intermittent carriers [33]. Third, atypical S. aureus isolates were detected e.g. isolates that were nuc negative and these could have been other species of coagulase positive staphylococci (e.g. S. argenteus [46]) misidentified as S. aureus. It is well documented that there is no single phenotypic test that can always accurately identify S. aureus and the detection of atypical isolates of S. aureus in this setting was reported before [12]. As such, we regarded the nuc negative isolates to be S. aureus because the Phoenix ID/100 system we used as a tie-breaker confirmed them as so. It is important to note that currently, the Expert Identification Systems such as the Phoenix ID/100 & Vitek2 GP Card (bioMérieux) cannot distinguish S. aureus from other species of coagulase positive staphylococci [46].

Conclusions

The nasopharyngeal carriage rate of S. aureus (MSSA/MRSA) in children in Eastern Uganda is high and comparable to estimates for adult patients at Mulago Hospital in Kampala. As high levels of MDR isolates were detected, outpatient treatment of Staphylococcus infections in children in Eastern Uganda might be difficult. As well, the detection of mupirocin resistant isolates is a cause for concern in that once mupirocin is introduced for Staphylococcus decolonization, it will likely spike a rapid increase in resistance in a low-income setting. As surveys of adult populations show a reduction in S. aureus nasal carriage rate and this is attributed to factors like improved personal hygiene, smaller families, etc. [1], health education of target populations on standard hygiene practices is necessary for MRSA control in Uganda. Lastly, S. aureus isolates of spa types t037, t064, t645 and t4353 are prevalent in Uganda and could be responsible for majority of staphylococcal infections in the country.
Abbreviations

IMHDSS: Iganga-Mayuge Health & Demographic Surveillance Site

MSSA: Methicillin Susceptible *Staphylococcus aureus*

MRSA: Methicillin resistant *Staphylococcus aureus*

MIC: Minimum inhibitory concentrations

SCCmec: Staphylococcal cassette chromosome mec

HLMup*: High-level mupirocin resistance

PCR: Polymerase chain reaction

PVL: Panton Valentine leukocidin

Spa: Staphylococcal protein A

SXT: Trimethoprim/sulfamethoxazole

NCBI: National Center for Biotechnology Information

Declarations

**Ethics approval and consent to participate**

This study was approved by the Higher Degrees Research and Ethics Committee of the School of Biomedical Sciences, Makerere University (Approval Number SBS194). The investigated bacteria were isolated during the studies of Kateete et al 2011 [8], Kateete et al 2013 [29], Seni et al 2013 [9, 27] and Rutebemberwa et al 2015 [10], which obtained prior approvals (REC REF 2011-183) from the Institutional Review Boards of the Schools of Medicine and Public Health at Makerere University, and the Uganda National Council for Science and Technology (Ref HS 1080). The parent studies obtained assent from parents/guardians on behalf of the children to participate in the study, as well as consent for sample storage and future use of stored samples in further studies.

**Consent for publication**
Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Authors’ contributions

DPK conceived the study, analyzed and interpreted the data, and wrote the first draft of the manuscript. ER & KK recruited the study participants and provided samples from which staphylococci were isolated. DPK, FB, BM & RM performed the microbiological procedures and molecular assays. BBA performed the PFGE and analysis. DPK & CFN supervised the study protocol. All authors read and approved the final manuscript.

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Additional Files

**Additional file 1: Table S1:** Spa Types and antibiotic susceptibility profiles of MSSA & MRSA from children ≤5 years in rural eastern Uganda.

**Additional file 2: Table S2:** General characteristics of *S. aureus* from children under 5 years of age at the IMHDSS, eastern Uganda.

**Additional file 3: Figure S1:** PFGE analysis of *S. aureus* showing 10 clusters (A-J) of isolates with similar profiles hence genetically related. This analysis confirmed clusters (C & D) with MRSA isolates of *spa* type t064. +, PVL-positive; -, PVL-negative

**Additional file 4: Table S3:** Distribution of *spa* types among *S. aureus* in Uganda

**Additional file 5: Figure S2:** The most frequent *spa* types for *S. aureus* clones circulating at IMHDSS (panel A), Mulago Hospital (panel B) & rural western Uganda (panel C). The predominant genotypes in each setting are indicated in a broken circle. This analysis shows that MRSA infections in Uganda are more likely to be associated with *spa* types t064, t037, t645 & t318.

Figures
Study flow chart illustrating the procedure we followed to identify S. aureus
Figure 2

The most frequent spa types for S. aureus clones circulating in Uganda

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Table-S1.pdf
Figure-S1.tiff
Table-S2.xlsx
Table-S3.pdf
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