Nasopharyngeal carriage, spa types and antibiotic susceptibility profiles of Staphylococcus aureus from healthy children less than 5 years in Eastern Uganda

CURRENT STATUS: ACCEPTED

BMC Infectious Diseases

David Kateete
Makerere University College of Health Sciences
davidkateete@gmail.com
ORCiD: 0000-0001-8075-3069

Benon B Asiimwe
Makerere University College of Health Sciences

Raymond Mayanja
Makerere University College of Health Sciences

Brian Mujuni
Makerere University College of Health Sciences

Freddie Bwanga
Makerere University College of Health Sciences

Christine F Najjuka
Makerere University College of Health Sciences

Karin Källander
Malaria Consortium

Elizeus Rutebemberwa
Makerere University College of Health Sciences

DOI: 10.21203/rs.2.10945/v5

SUBJECT AREAS
Infectious Diseases

KEYWORDS
Staphylococcus aureus; Carriage; MSSA/MRSA; Urban/rural; Iganga/Mayuge
districts; Multidrug resistant; Genotyping
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 identify S. aureus lineages that cause infection in Uganda.

Methods: Nasopharyngeal samples from 742 healthy children less than 5 years residing in the Iganga/Mayuge Health and Demographic Surveillance Site in Eastern Uganda were processed for isolation of S. aureus. Antibiotic susceptibility testing based on minimum inhibitory concentrations (MICs) was determined by the BD PhoenixTM system. Genotyping was performed by spa and SCCmec 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 isolates were susceptible to rifampicin, vancomycin and linezolid. Moreover, all MRSA were susceptible to vancomycin, linezolid and clindamycin. Compared to methicillin susceptible S. aureus (MSSA) isolates (68.8%, 99/144), MRSA isolates were more resistant to non-beta-lactam antimicrobials – trimethoprim/sulfamethoxazole 73.3% (33/45) vs. 27.3% (27/99) p<0.0001; erythromycin 75.6% (34/45) vs. 24.2% (24/99) p<0.0001; chloramphenicol 60% (27/45) vs. 19.2% (19/99) p<0.0001; gentamicin 55.6% (25/45) vs. 25.3% (25/99) p=0.0004; and ciprofloxacin 35.6% (16/45) vs. 2% (2/99) p<0.0001.

Furthermore, 42 MRSA (93.3%) were multidrug resistant (MDR) and one exhibited high-level resistance to mupirocin. Overall, 61 MSSA (61.6%) were MDR, including three mupirocin and clindamycin resistant isolates. Seven spa types were detected among MRSA, of which t037 and t064 were predominant and associated with SCCmec types I and IV, respectively. Fourteen spa types were detected in MSSA which consisted mainly of t645
Conclusions: S. aureus carriage rate in healthy children in Eastern Uganda is high and comparable to rates for hospitalized patients in Kampala. 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 the 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 individuals in Africa 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 the community especially among 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 in healthy children in rural Eastern Uganda were found to be high at 58.6% (89/152), 15% (23/152) and 11% (16/152), respectively [10]. These
organisms alongside *S. aureus*, are the main causes of bacterial pneumonia [10], the leading cause of death in children less than 5 years of age [11]. In a study by Rutebenderwa et al [10], the antibiotic susceptibility profiles and carriage rates of *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* in children less than 5 years residing in the Iganga/Mayuge Health and Demographic Surveillance Site (IMHDSS) in Eastern Uganda, was determined. However, the rates and characteristics of *S. aureus*, a frequent cause of infections in children, were not documented. Therefore, this follow-up study aimed to determine the carriage rate and antibiotic susceptibility patterns of *S. aureus* from children in Eastern Uganda. The study also aimed to identify *S. aureus* lineages that can cause infection in community and/or health care settings in Uganda. Due to frequent interaction between health care workers from Uganda’s only national referral hospital (Mulago in Kampala) and community members, we hypothesized that *S. aureus* isolates from the hospital and community will generally have the same genetic background.

**Methods**

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

This cross-sectional study was conducted between February and October 2011, nested in studies/projects that investigated the pneumococcal carriage rate in healthy children at the IMHDSS. The IMHDSS is located in Iganga and Mayuge districts in Eastern Uganda, towards the border between Uganda and Kenya. Prior to the current study on *S. aureus*, there was a pilot study in 2008 that investigated pneumococcal carriage in children at the IMHDSS [10]. Subsequently, a follow-up study was conducted in 2011, in which ~1,300 children less than 5 years were screened for pneumococcal carriage [unpublished observations]. The 2011 follow-up study had several objectives one of which was investigating the integrated community case management of malaria and pneumonia in
communities and appropriate treatment of pneumonia symptoms in children less than five years of age [36]. The 2011 study also investigated pneumococcal carriage in children at the IMHDSS [unpublished observations], and the current study on S. aureus was nested in this arm of the project, in which samples from 742 children (of ~1,300 children investigated for pneumococcal carriage) were concurrently processed for isolation of S. aureus.

The procedure for isolation and identification of S. aureus is depicted in Figure 1. 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 testing, (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 methods were subjected to PCR-detection of the nuc gene [12]. Isolates were considered to be S. aureus if a 270 base pair fragment was identified on agarose gel electrophoresis. 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 and BD Phoenix 100 ID/AST) were excluded from further analysis.

**Antibiotic susceptibility testing**

Antibiotic susceptibility testing was performed based on minimum inhibitory concentrations (MICs) using the BD Phoenix 100 ID/AST expert system. Multidrug resistance (MDR) was defined as isolates resistant to three or more classes of antimicrobials.
**Genotyping and other molecular procedures**

To determine the *S. aureus* 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 the PCRs. To obtain *spa* types, 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 To identify *S. aureus* lineages that are likely to cause infection in Uganda, we compared *spa* types for *S. aureus* at the IMHDSS to previously described *spa* types at Mulago Hospital in Kampala [9], as well as pastoral communities in Western Uganda [17, 18]. Further, SCCmec types among MRSA isolates were determined by using a previously published PCR protocol [19]. To detect the PVL-encoding genes (*lukS-PV* and *lukF-PV*), isolates were screened by PCR as previously described [8, 9]. For isolates with frequently occurring *spa* types, pulse field gel electrophoresis (PFGE) analysis was performed according to Asiimwe et al [17]. Briefly, isolates were subjected to Sma I 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. [20]. 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 [20]. In all procedures 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 encoding genes was done for selected isolates and confirmed by BLAST-searching at NCBI https://blast.ncbi.nlm.nih.gov/Blast.cgi

Statistical analysis was performed with the SPSS statistical software (version 14.0, Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant. We used the Chi Square test to determine the relationship between the frequencies of spa types, SCCmec types, and setting.

Results

**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). Forty five (31.3%, 45/144) of the isolates were confirmed to be MRSA yielding a carriage rate of 6.1% (45/742). The MSSA carriage rate was 13.3% (99/742).

**Spa Types, resistance patterns and antibiotypes**

In this study, all the isolates were susceptible to rifampicin, vancomycin and linezolid, while all the MRSA were susceptible to vancomycin, linezolid and clindamycin (Tables S1 & S2). Compared to MSSA isolates (68.8%, 99/144), MRSA isolates were more resistant to non-beta-lactam antimicrobials i.e. trimethoprim/sulfamethoxazole (co-trimoxazole) 73.3% (33/45) vs. 27.3% (27/99) [p<0.0001], erythromycin 75.6% (34/45) vs. 24.2% (24/99) [p<0.0001], chloramphenicol 60% (27/45) vs. 19.2% (19/99) [p<0.0001], gentamicin 55.6% (25/45) vs. 25.3% (25/99) [p=0.0004] and ciprofloxacin 35.6% (16/45) vs. 2% (2/99) [p<0.0001]. One MRSA isolate (K2283) exhibited high-level mupirocin resistance (HLMup′) while 42 (93.3%, 42/45) were MDR including the mupirocin resistant isolate (Tables S1 &
S2). The proportion of PVL+ MSSA and PVL+ MRSA was 9/99 (9.1%) and 19/45 (13.2%), respectively. All PVL+ MRSA isolates were MDR and generally the presence of PVL genes was associated with the MDR phenotype (P=0.0332). Seven 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 (Tables S1 & S2). Spa types t037 and t064 were significantly associated with MRSA and SCCmec types I & IV respectively, with t037 exclusively occurring in MRSA (Table 1). A general description of the number of clusters observed on PFGE analysis and diversity of the collection is shown in Figure S1.

Table 1: Frequency of spa types among S. aureus isolates from children in IMHDSS, Eastern Uganda

| Spa type | MDR (%) | MESSA | MRSA | Total |
|----------|---------|-------|-------|-------|
|          | Yes     | No    | Frequency | RF | Frequency | RF | Frequency |
| t064     | 10 (66.7) | 05 (33.3) | 06 | 6.1 | 9 | 20 | 15 |
| t645     | 06 (55.4) | 05 (45.5) | 11 | 11.1 | 0 | 0 | 11 |
| t4353    | 06 (60) | 04 (40) | 9 | 9.1 | 1 | 2.2 | 10 |
| t002     | 06 (85.7) | 01 (14.3) | 6 | 6.1 | 1 | 2.2 | 7 |
| t037     | 06 (85.7) | 01 (14.3) | 0 | 0 | 7 | 15.6 | 7 |
| t078     | - | - | 2 | 2 | 0 | 0 | 2 |
| t355     | - | - | 1 | 1 | 1 | 2.2 | 2 |
| t3092    | - | - | 1 | 1 | 1 | 2.2 | 2 |
| t12939   | - | - | 0 | 0 | 1 | 2.2 | 1 |
| t3662    | - | - | 1 | 1 | 0 | 0 | 1 |
| t318     | - | - | 1 | 1 | 0 | 0 | 1 |
| t1456    | - | - | 1 | 1 | 0 | 0 | 1 |
| t10394   | - | - | 1 | 1 | 0 | 0 | 1 |
| t1476    | - | - | 1 | 1 | 0 | 0 | 1 |
| t2168    | - | - | 1 | 1 | 0 | 0 | 1 |
| t213     | - | - | 1 | 1 | 0 | 0 | 1 |
| Unknown  | - | - | 4 | - | 0 | 0 | 4 |
| NT       | - | - | 17 | - | 4 | - | 21 |
| ND       | - | - | 35 | - | 20 | - | 55 |
| Total    | - | - | 99 | - | 45 | - | 144 |

The predominant spa types are depicted in bold font. RF denotes Relative Frequency (%) i.e., the number of times that the event (i.e. spa lineage) occurred, divided by the total in that category. NT, not type-able; ND, not determined.

Ninety nine (68.8%, 99/144) isolates were MSSA as they were cefoxitin susceptible and
mecA negative (Tables S1 & S2). The percentage and proportion of MSSA isolates resistant to penicillin, tetracycline, cotrimoxazole, erythromycin, gentamicin and chloramphenicol are 78.8% (78/99), 79.8% (79/99), 27.3% (27/99), 24.2% (24/99), 25.3% (25/99) and 19.2% (19/99), respectively. Three (3%, 3/99) MSSA isolates (K277-1, K251 and 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). Fourteen spa types were detected among MSSA, of which t645 (11.1%, 11/99), t4353 (9.1%, 9/99), t064 (6.1%, 6/99) and t002 (6.1%, 6/99) were predominant (Tables S1 & S2). Spa type t645 exclusively occurred in MSSA (Table 1). There were other spa types that exclusively occurred in MSSA e.g. t078, t3662, t318, t1456, t10394, t1476, t2168 and t213 (Table 1) but their frequencies were too low (i.e. ≤2 occurrences) to allow meaningful analysis.

Table 2 summarizes the antibiotypes and their relationship with spa and SCCmec types. Overall, a total of 38 and 28 antibiotypes were detected among MSSA and MRSA isolates, respectively. 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 and MRSA and their relationship with spa types
| Antibiotype | Resistance profile | No. isolates showing this pattern (%) | Major spa types (frequency) |
|------------|--------------------|--------------------------------------|----------------------------|
| S1         | PEN-TET            | 17 (17.2)                            | t064 (4), t4353 (3), t645 (2), t355 (1) |
| S2         | PEN-TET-ERY       | 11 (11.1)                            | t002 (3), t645 (1), t078 (1), t4353 (1), t2168 (1) |
| S3         | PEN-TET-GEN       | 9 (9.1)                              | t318 (1), t213 (1), t1476 (1) |
| S4         | PEN                | 6 (6.1)                              | t002 (1), t645 (1), t4353 (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), t1456 (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), t10394 (1) |
| S12        | PEN-TET-ERY-CHL   | 2 (2)                                | t064 (1), t3092 (1) |
| S13        | PEN-SXT           | 2 (2)                                | t4353 (1) |
| S14        | PEN-TET-SXT-ERY   | 2 (2)                                | t645 (1) |
| S38        | - (Pan-susceptible) | 3 (3)                | t064 (1) |

**MSSA**

| Antibiotype | Resistance profile | No. isolates showing this pattern (%) | Major spa types (frequency) |
|------------|--------------------|--------------------------------------|----------------------------|
| R1         | PEN-FOX-TET-SXT-ERY-CHL-GEN | 7 (15.6) | - (4), t064 (3) |
| R2         | PEN-FOX-TET-SXT-ERY-CHL-GEN-CIP | 4 (9) | - |
| R3         | PEN-FOX-TET-SXT-ERY-CHL-GEN | 4 (9) | - |
| R4         | PEN-PEN-TET-SXT-ERY | 3 (6.7) | t002 (1), t064 (1) |
| R5         | PEN-FOX-TET-SXT-ERY-CIP | 3 (6.7) | t064 (1) |
| R6         | PEN-FOX-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, gentamicin; 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 the genotypes of *S. aureus* isolates were compared with previously characterized isolates in Uganda, it was observed that the *spa* types detected at IMHDSS were previously reported from Mulago Hospital in Kampala, but slightly different from rural Western Uganda (Figure S2). The number of isolates from the other sites (i.e. Mulago Hospital and rural Western Uganda) were as follows: 105 (Mulago Hospital, 64 & 41 from Seni et al [9] & Kateete et al [8], respectively) and 73 (rural Western Uganda) [18]. Of these, MRSA isolates were 113 (65 Mulago Hospital and 48 rural Western Uganda). Overall, 40 *spa* types accounted for MSSA/MRSA clones, of which t645, t064, t4353, t002, t318, t037, t355, t084, t3772, t127 and t186 were predominant (Figure 2 & Table S3). The frequent *spa* lineages in each of the sites were t064, t645, t4353, t002 and t037 (IMHDSS); t645, t4353, t064, t084, t355, t3772 and t4609 (Mulago Hospital); and t318, t064, t645, t186, t11656, t127, t786 and t2771 (rural Western Uganda) (Figure S2). *Spa* type t037 exclusively occurred in MRSA and it was detected only at IMHDSS and Mulago Hospital. On the other hand, *spa* types t645 and t4353 occurred in all the three sites and they were significantly associated with MSSA (Table S3 & Figure 2). When isolates from the three sites were analyzed, *spa* types t4353, t002 and t355 were neither associated with MRSA or MSSA. Interestingly, *spa* type t064 that was significantly associated with MRSA at IMHDSS was not associated with MRSA at Mulago Hospital (Table S3).

**Discussion**

*Staphylococcus aureus* infection in Uganda was first reported at Mulago 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 there is breach of the
body barriers (e.g. skin) or when the immune system is weakened. In this study, the *S. aureus* nasopharyngeal carriage rate (19.4%) in 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 [8, 9]. Further, 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]). However, the study populations are diverse making direct comparisons difficult.

Several factors could be responsible for the high MRSA carriage rate in Ugandan children e.g. carriage by parents and previous exposure to antibiotics as one third of the children at the IMHDSS had prior antibiotic exposure particularly ampicillin and co-trimoxazole. Overcrowding is another factor that could have contributed to the observed high carriage rates in children as the pilot study at the IMHDSS found the median number of household members per bedroom (as a measure of crowding) to be four [10]. Large households of ≥5 members are associated with high *S. aureus* nasal carriage rates [1]. The demographic characteristics typical of the children sampled were described previously [10, 36]. Most children at the IMHDSS live in rural areas and their primary source of water is spring water and well water. By 2008, only 1% of the children had access to piped water and the median number of children per household is 2.

In this study, the proportion of MSSA isolates resistant to penicillin and tetracycline was 78%-79.8%, while resistance to co-trimoxazole, 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 [37]. 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 the rate reported in this study (91%). Furthermore, MRSA isolates in this study were MDR and this is also consistent with various reports on MRSA [32, 38]. However, it is important to note that the resistance profiles among countries would depend on the different practices of antibiotic use between the countries, antibiotic stewardship and enforcement of infection control practices. Overall, clindamycin, rifampicin, vancomycin and linezolid were the most effective antibiotics in this study, in contrast to findings from Ethiopia where gentamicin and ciprofloxacin were the most effective among isolates from primary school children [34].

Mupirocin is a relatively new drug that was introduced in Uganda in the 2000s [39]. It is used as a topical antibiotic to treat skin infections (e.g. impetigo, inflammation of hair follicles, minor skin infections, boils) caused by *S. aureus* and *Streptococcus pyogenes*. Mupirocin is also commonly used for nasal decolonization of MSSA and MRSA in patients and health care workers in the developed countries [5, 32, 37]. We have reported a high rate (4%) of mupirocin resistance (high-level) and this is consistent with the reported rates for this drug in Africa (5%-50%) [40]. The detection of mupirocin resistance in Uganda is worrying as MRSA decolonization is generally not a common practice in this country. As mupirocin use in decolonization regimens leads to rapid emergence of resistance [32], its frequent use in Uganda for eradication of *Staphylococcus* colonization will likely spike an increase in resistance [40].

It is interesting to note that attempts to type staphylococci in Uganda date back to 1958 when phage typing was used to characterize *S. aureus* isolates at Mulago Hospital [21]. However, the typing of MRSA in Uganda was first reported in 1992 [41] (by multi-locus enzyme electrophoresis). Overall, this study has shown that *S. aureus* strains of spa types t645, t064, t4353, t002, t318, t037, t355, t084, t3772 and t127 are prevalent in Uganda,
and they appear be common in Africa [35, 42, 43]. Consistent with this, a systematic review of the global distribution of spa types revealed that t064 and t037 are the most prevalent in Africa with t037 being exclusively associated with MRSA isolates [44]. The exception is t4353, which appears to be common in Uganda and other settings in East Africa [9, 18, 35] but rare in other African countries [42, 48]. Consequently, 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 with ST-121 [45]. It is important to note that, while spa type t037 is frequently associated with hospital-associated MRSA strains, it has mainly been associated with SCCmec type III [46, 47] though other investigators have associated it with SCCmec types I & IVc [46].

There were certain limitations in this study. First, we used the http://spatyper.fortinbras.us/ server for identification of spa types as it is freely accessible but it is not the official database for determining spa types. However, several investigators [48-51] have used the http://spatyper.fortinbras.us/ server to accurately determine spa types. Generally, PCR genotyping approaches are prone to errors in interpretation and the molecular marker itself can yield results that may depict homoplasy events and/or convergent evolution. This, and the fact that our sample size for MRSA isolates was relatively small, will require larger studies with robust approaches such as whole genome sequencing. Second, we were not able to type all the isolates recovered from the children. Because of this, and the fact that few studies have performed spa typing of S. aureus isolates in Uganda, the reported frequencies and associations should be taken with caution as frequencies could change with genotyping of more isolates. We also lacked information on clonal complexes based on MLST. Although it was inappropriate
to relate isolates from children and adults, our aim was to describe the population structure of these isolates without drawing inferences on epidemiological linkages. Third, we used a cross-sectional design with a single sampling point to classify children as carriers implying that some children classified as non-carriers might have been intermittent carriers [33]. Furthermore, atypical S. aureus isolates were detected e.g. coagulase positive isolates that were nuc negative, and these could have been other species of coagulase positive staphylococci (e.g. S. argenteus [52]) misidentified as S. aureus. However, 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]. The Expert Identification Systems such as Phoenix ID/100 and Vitek2 GP Card (bioMérieux) may also not differentiate atypical S. aureus [52]. As such, we regarded nuc negative isolates to be S. aureus because the Phoenix ID/100 system used as a tie-breaker confirmed their identity.

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 S. aureus infections in children in Eastern Uganda might be difficult. Moreover, 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. 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 [1]. Hence, 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 (MRSA), and t645 & t4353 (MSSA) 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 \textit{Staphylococcus aureus}

MRSA: Methicillin resistant \textit{Staphylococcus aureus}

MLST: Multi-locus sequence typing

MIC: Minimum inhibitory concentrations

SCC\textit{mec}: Staphylococcal cassette chromosome \textit{mec}

HLMup\textsuperscript{f}: 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

\textbf{Ethics approval and consent to participate}

This study was approved by the Makerere University School of Biomedical Sciences Research and Ethics Committee (Approval Number SBS194), and by the Institutional Review Boards of the Schools of Medicine and Public Health at Makerere University (REC REF 2011-183) 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.

\textbf{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**

David Kateete is an editorial board member for BMC Infectious Diseases

**Funding**

This work was supported through funding from GlaxoSmithKline (gsk)’s Trust in Science scheme, and through postdoctoral research support (to DPK) from Carnegie Cooperation (NGAAI&II) through the Directorate of Research and Graduate Training, Makerere University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Authors' contributions**

DPK conceived the study, analyzed and interpreted the data, and wrote the first draft of the manuscript. ER and 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 and CFN supervised the study protocol. All authors read and approved the final manuscript.

**Acknowledgements**

We thank the mothers and/or guardians for the children for accepting to participate in the study. We also thank the staff and management of the Clinical Microbiology and Molecular Biology Laboratories of Makerere University College of Health Sciences for technical assistance.

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Additional Files

Additional file 1: Table S1: Spa types and antibiotic susceptibility profiles of MSSA and MRSA from children less than 5 years in rural eastern Uganda.

Additional file 2: Table S2: General characteristics of S. aureus from children less than 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: Frequency of spa types among S. aureus in Uganda

Additional file 5: Figure S2: The most frequent spa types among S. aureus from the IMHDSS (panel A), Mulago Hospital (panel B) and rural western Uganda (panel C). Asterisks indicate frequencies for common spa types in Uganda and their association with either MRSA or MSSA. This analysis showed that MRSA infections in Uganda are more likely to be associated with spa types t064, t037, t645 and t318.

Figures
Figure 1

Study flow chart illustrating the procedure for the isolation and identification of S. aureus
The most common S. aureus spa types identified in Uganda. Asterisks indicate frequencies for common spa types in Uganda and their association with either MRSA (t037, t064) or MSSA (t645, t4353).

Supplementary Files

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

Table-S1.pdf
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Figure-S2[1].jpg
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