Nasal carriage of meticillin-resistant *Staphylococcus aureus* among children living with HIV attending Infectious Diseases Clinics in Kano, Nigeria

Aisha Habib Sadauki a,*, Abdulhakeem Abayomi Olorukooba b, Muhammad Shakir Balogun c, Mahmood Muazu Dalhat c, Hyelshilni Waziri c, Mukhtar Muhammad Abdulaziz d, Chukwuma David Umeokonkwo c,e, Fatimah Hassan-Hanga f, Kabir Sabitu b

a Nigeria Field Epidemiology and Laboratory Training Program, Nigeria Centre for Disease Control, Federal Ministry of Health, Nigeria  
b Department of Community Medicine, Ahmadu Bello University Zaria, Nigeria  
c African Field Epidemiology Network, Abuja, Nigeria  
d Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria  
e Department of Community Medicine, Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria  
f Department of Paediatrics, Aminu Kano Teaching Hospital, Bayero University, Kano, Nigeria

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SUMMARY

**Background:** Children living with HIV (CLWH) are at risk of colonisation and infection with meticillin-resistant *Staphylococcus aureus* (MRSA). All *S. aureus* isolates from CLWH with bloodstream infections in Kano were MRSA.

**Aim:** To estimate the prevalence of nasal colonisation with *S. aureus* and MRSA in CLWH in Kano State and to determine associated risk factors.

**Methods:** A cross-sectional study was performed in the infectious diseases clinics of two public hospitals in Kano involving 214 CLWH/caregiver pairs. Children were selected from clinic registers by simple random sampling and an interviewer-administered questionnaire used to elicit factors associated with MRSA carriage from the caregivers. Clinical records were reviewed for patients’ medical histories. Standard laboratory techniques were used to isolate *S. aureus* from nasal swabs collected from CLWH. MRSA was detected using the cefoxitin disc diffusion method and PCR for *mecA* gene detection. We measured the prevalence of *S. aureus* and MRSA carriage in the CLWH and calculated adjusted odds ratios (AOR) for factors associated with MRSA.

**Results:** Nasal *S. aureus* carriage in CLWH was 18.7% (40/214). Cefoxitin disc diffusion identified 6/214 (2.8%) of CLWH were MRSA carriers, while PCR identified that 9/214 (4.2%) of CLWH were MRSA carriers. Recent hospitalisation (AOR: 61.04; 95% CI: 9.01–413.38) and recent antibiotic therapy (AOR: 7.52; 95% CI: 1.07–52.95) were independent risk factors for MRSA colonisation.

**Conclusions:** The rate of MRSA nasal colonisation among CLWH in Kano was similar to that reported in other studies in Africa. Infection prevention and control measures including
Introduction

Globally, antimicrobial resistance accounts for an estimated 700,000 deaths annually [1]. Meticillin-resistant Staphylococcus aureus (MRSA) is one of the major antimicrobial-resistant pathogens. [2]. An estimated 670,000 antimicrobial-resistant infections occur in Europe annually resulting in 33,000 deaths [3]. In 2019, 15.5% of S. aureus isolates reported from European countries were MRSA [4]. In the United States, each year approximately 2 million people are infected with antimicrobial-resistant bacteria leading to over 23,000 deaths annually. Of the 23,000 annual deaths attributed to antimicrobial-resistant bacteria in the United States, MRSA contributes 19,000 [5]. There is limited information on the burden of antimicrobial resistance in Africa due to the lack of any available data from some countries, insufficient surveillance data, limited data from settings other than hospitals, urban areas or febrile patients, and inadequate laboratory facilities to detect and track antimicrobial resistance. [6,7]

Data obtained from a study in African countries showed that the median prevalence of resistance to oxacillin (an antibiotic used in laboratories to test for meticillin resistance) in S. aureus isolated from febrile patients was 13.4% for West Africa and 8.0% for East Africa [6]. In hospitalised children living with HIV (CLWH) who have bloodstream infections in Kano, S. aureus accounted for 4/19 (21%) of the isolates [8]. All four isolates were resistant to oxacillin [8].

The risk of both MRSA colonisation and infection are increased in children, the elderly, athletes, intravenous drug users, those living with HIV and those with frequent contact with healthcare [9]. CLWH are therefore a high-risk group for MRSA colonisation and infection. Planning and implementing an appropriate antimicrobial policy for the treatment of staphylococcal infections in the HIV positive patients is a key strategy for the control of MRSA infections in this population [10]. To formulate such guidelines, data are required on the prevalence, pattern and antimicrobial susceptibility of S. aureus organisms present in CLWH. This study was carried out in CLWH in Kano State, Nigeria to measure the prevalence of nasal S. aureus colonisation, to investigate the antimicrobial sensitivity of S. aureus colonising anterior nares of the CLWH and to measure the prevalence of MRSA carriage and associated risk factors.

Methods

Study setting

The study took place in Kano State North-western Nigeria. The prevalence of HIV infection in Kano State was 0.5% according to the Nigeria HIV/AIDS Indicator and Impact Survey 2018 [11]. There are four public facilities with Paediatric Infectious Diseases Clinics (PIDCs) run by paediatric specialists offering care to CLWH in Kano. They include the Aminu Kano Teaching Hospital (AKTH), a tertiary facility, Murtala Muhammad Specialist Hospital (MMSH), Muhammad Abdullahi Wase Specialist Hospital (MAWSH), and Hasiya Bayero Paediatric Hospital (HBPH) which are secondary health facilities.

The study took place in the infectious disease clinics of HBPH and AKTH.

Aminu Kano Teaching Hospital (AKTH) is a 500-bed hospital that offers tertiary healthcare to people in Kano and the neighbouring states [12]. In March 2019, there were 373 active patients aged 0–14 years receiving care in the Infectious Diseases clinic. This clinic treated CLWH every working day of the week. Hasiya Bayero Paediatric Hospital (HBPH) is a secondary health facility specifically for children. As of March 2019, there were 237 active patients aged 0–14 years receiving care in the Infectious Diseases clinic of HBPH. This clinic took place twice a week. Medical records of patients in both clinics were stored on electronic databases.

Study design

Cross-sectional

Study population

CLWH/caregiver pairs attending the PIDCs in AKTH and HBPH were included in the study. Children in whom a diagnosis of HIV was yet to be confirmed by Polymerase Chain Reaction (PCR) test or antibody test as applicable and new patients enrolled after 31st March 2019 were excluded from the study.

Ethical approval

The study protocol was approved by the Research Ethics Committee of the Aminu Kano Teaching Hospital, Kano, Nigeria with approval number NHREC/21/08/2008/AKTH/EC/2425. Written informed consent was obtained from the parents or guardians of the participants. In addition, verbal assent was obtained from the participants who were 10 years or older. Patient identifiers were removed from the data and all data obtained were kept confidential. Data was stored on a password-protected device and encrypted before electronic transfer.

Study period

The study took place from April to June 2019. Sample size: Sample size was estimated using the formula for single proportion: \( n = \frac{Z^2 \cdot p \cdot q}{d^2} \), at a precision of 5%, prevalence of S. aureus carriage among CLWH in a previous study of 22.1% and level of significance of 5% [13,14]. Sample size was adjusted for a finite population and increased by 6% based on the non-response rate of 6% from a previous study [15]. The estimated sample size was 197 children. To calculate the number of children to be recruited from the Infectious Diseases clinics in AKTH and
HBPH, proportional allocation was used. This was calculated as AKTH 120 children and HBPH 77 children.

**Sampling technique**

A multi-stage sampling technique was employed. In the first stage, two of the four PIDCs, AKTH and HBPH were chosen using simple random sampling without replacement. In the second stage, a list of hospital numbers of all active patients aged 0–14 years was created in Microsoft Excel 2016® spreadsheets from each of the clinics selected in stage one and this was used as the sampling frame. Simple random sampling without replacement was used to select children aged 0–14 years from the sampling frame. In the Microsoft Excel 2016® spreadsheet, the hospital numbers of the children aged 0–14 years were placed in column A titled "Hospital number". In column B, which is titled "Random number", a list of random numbers between 0 and 1 was generated using the "RAND" function in Microsoft Excel 2016®. In column C, which is titled "Random sample" a random sample of the hospital numbers without duplication was generated by typing the formula “=INDEX (A$A2:A$373,RANK(B2,A$B$2:A$B$373),1). The clinic booking register was used to schedule interviews with the randomly selected children and their caregivers on their appointment days.

**Data collection**

We used an interviewer-administered structured questionnaire to obtain socio-demographic characteristics and exposure factors for nasal MRSA colonisation in the children. The questionnaire was adapted from previous studies [16,17] and pre-tested at the PIDC in MMSH. A separate section in the questionnaire was used to document clinical information such as viral load, antibiotic prescriptions in the previous 3 months and anti-retroviral treatment history from the patients’ records. Two medical officers were recruited and trained to administer the questionnaire to caregivers, to review hospital records. Two medical officers were recruited and trained to administer the questionnaire to caregivers, to review hospital records and to obtain nasal swabs from the children.

**Specimen collection and handling**

A cotton wool swab was moistened with normal saline and used to swab both anterior nares of all enrolled children. Nasal swabs were transported to the laboratory in Stuart’s medium (Micropoint Bioscience, Inc) at ambient temperature. Within 2 hours of collection, swabs were inoculated directly onto Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, UK) [18]. The results were interpreted according to CLSI 2018 guidelines [18].

PCR was conducted on all S. aureus isolates for the detection of mecA. The protocol for PCR for mecA is summarised as follows. Primer sequences used for methicillin-resistant *Staphylococcus aureus* (MecA) gene primer were MecA-F5' GTGGAATTGGCAATACACC-3' and MecA-R 5' AGTTCTGCAGTACCGGAT-3' (Biomers.net, Germany). 20 μl was prepared for each of the samples. The PCR set up consisted of 2 μl of DNA extract, 4 μl of primers (forward and reverse), 16 μl of distilled water, all in a tube. All tubes were sealed and centrifuged then transferred into the PCR machine. Amplification was done with the following protocol: initial denaturation at 94°C for 5 minutes, then 35 cycles of denaturation at 94°C for 40
seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 60 seconds and final extension at 72 °C for 5 minutes. Each amplification run contained one negative control. After amplification, electrophoretic separation of PCR products was performed on 1.5% agarose gel stained with ethidium bromide, and visualised by UV illumination. For quality control, a known MRSA DNA-positive control (mecA positive strain ATCC 33591) and a negative control using RNA/DNA free water were run along with the samples during each PCR run. The positive control always gave a positive band at 536 bp while the negative control always showed no band. Samples corresponding to electrophoretic lanes with band at a level equivalent to 536 bp on the DNA molecular weight marker were documented as positive for mecA DNA. The samples that did not meet this criterion were considered negative.

**Data analysis**

The data were entered into Microsoft Excel 2016 ® spreadsheets and imported into IBM® Statistical Package for Social Sciences ®(SPSS) version 28 and analysed [19]. The proportion of children living with HIV who had S. aureus and MRSA carriage was calculated. The relationship between the MRSA carriage and the various exposure variables was examined using odds ratio. Variables with a P-value of ≤ 0.2 on bivariate analysis were entered into a multivariate regression model to adjust for confounding and adjusted odds ratios were calculated.

**Results**

A total of 220 CLWH/caregiver pairs were approached to take part in the study. Six pairs were unavailable or declined to take part. The response rate was 214/220 (97.3%). The mean (±S. D) age of the CLWH was 8.9 (±3.8) years. The median (range) monthly income of the households of the respondents was 30,000 Nigerian Naira (500–505,000) (1 Nigerian Naira = approx. 0.0024 USD March 2022). Table 1 describes the socio-demographic characteristics of the CLWH/caregiver pairs. In terms of clinical characteristics, 203/214 (94.9%) and 10/214 (4.7%) were in WHO HIV clinical stages 1 and 2 respectively, 208/214 (97.2%) were being treated with HAART (Highly active

### Table 2

Relationship between household characteristics and nasal MRSA colonisation among children living with HIV attending clinics in Kano State, Nigeria (n = 214).

| Exposure                                                | Positive | Negative | cOR (95% C.I) | P-Value |
|---------------------------------------------------------|----------|----------|---------------|---------|
| Primary caregiver’s age (years)                         |          |          |               |         |
| < 30                                                     | 2        | 40       | 1.17 (0.24–5.89) | 0.84    |
| ≥ 30                                                     | 7        | 165      | 1             | -       |
| Primary caregiver’s educational status                  |          |          |               |         |
| Informal                                                | 2        | 57       | 0.74 (0.15–3.68) | 0.71    |
| Formal                                                  | 7        | 148      | 1             | -       |
| Average monthly household income                         |          |          |               |         |
| < 50,000 Nigerian naira                                 | 7        | 15       | 1.25 (0.25–6.21) | 0.78    |
| ≥ 50,000 Nigerian naira                                 | 2        | 54       | 1             | -       |
| Number of people in the household                       |          |          |               |         |
| < 5                                                     | 1        | 28       | 0.79 (0.10–6.56) | 0.83    |
| ≥ 5                                                     | 8        | 177      | 1             | -       |
| Number of people that sleep in child’s bedroom          |          |          |               |         |
| < 5                                                     | 6        | 159      | 0.58 (0.14–2.40) | 0.45    |
| ≥ 5                                                     | 3        | 46       | 1             | -       |
| Health worker in the household                          |          |          |               |         |
| Yes                                                     | 1        | 24       | 0.94 (0.11–7.87) | 0.96    |
| No                                                      | 8        | 181      | 1             | -       |
| History of hospitalisation in a household member        |          |          |               |         |
| Yes                                                     | 3        | 83       | 0.73 (0.17–3.02) | 0.67    |
| No                                                      | 6        | 122      | 1             | -       |
| History of contact with domestic animals                 |          |          |               |         |
| Yes                                                     | 4        | 100      | 0.84 (0.21–3.21) | 0.80    |
| No                                                      | 5        | 105      | 1             | -       |
antiretroviral therapy) and 162/214 (75.7%) were on SMP-TMX prophylaxis. None of the children had a chronic skin disorder or had undergone surgery in the previous year, while 20/214 (9.3%) of the respondents had been hospitalised in the previous year.

S. aureus colonisation was detected in 18.7% (40/214) of the participants. MRSA colonisation was found in 2.8% (6/214) of the participants using the cefoxitin disc diffusion method and 4.2% (9/214) using PCR for mecaA gene detection. All six isolates classified as MRSA using the cefoxitin screening method were positive for the mecaA gene. The antimicrobial sensitivities of the 40 S. aureus isolates was 3/40 (7.5%) for penicillin, 7/40 (17.5%) for SMP-TMX, 32/40 (80.0%) for ciprofloxacin, 37/40 (92.5%) for gentamicin and 39/40 (97.5%) for mupirocin. All the S. aureus isolates identified as MRSA were resistant to 3 or more classes of antibiotics. Only one of the MRSA strains was sensitive to SMP-TMX. Figure 1 shows the antibiotic sensitivity patterns of the forty S. aureus isolates.

On bivariate analysis there was no significant relationship between household characteristics and MRSA colonisation in CLWH (Table 2). Clinical characteristics such as history of hospitalisation in the preceding year (OR: 27.29; CI: 6.16\textasciitilde120.87) and history of antimicrobial use within the previous three months (OR: 7.58; CI: 1.92\textasciitilde29.92) were significantly associated with nasal carriage of MRSA (Table 3). A logistic regression was performed to ascertain the effects of gender, viral suppression, history of hospitalisation in the previous year and history of antibiotic use in the last three months on the likelihood that study participants had MRSA nasal colonisation. The logistic regression model was statistically significant, $\chi^2 = 34.6$, $P < 0.001$. The model explained 51.7% (Nagelkerke R$^2$) of the variance in MRSA nasal colonisation and correctly classified 97.0% of cases. Children that had received antibiotics in the last three months were 7.5 times more likely to have nasal MRSA colonisation than those who had not. Children who had been hospitalised in the previous year were 61.0 times more likely to have nasal MRSA colonisation than those who had not been hospitalised (Table 4).

**Discussion**

S. aureus carriage differs depending on location, ethnicity, sex, anatomical site, population being studied, and the presence of pre-existing conditions [20,21]. S. aureus nasal colonisation among CLWH in the present study was 18.7%. Carriers of S. aureus are at risk of staphylococcal infections from autoinoculation and may also serve as sources of infection to others [20]. The proportion of nasal S. aureus carriers in the present study is similar to the proportion of 22% reported among CLWH in Ghana [14]. A similar prevalence of 20.4% was reported among CLWH in South Africa [22]. Lemma et al. also reported nasal S. aureus carriage of 17% among CLWH in Ethiopia [16]. Nasal carriage of S. aureus among CLWH in Botswana was 55% [23]. The large discrepancy between the carriage rates reported in Botswana and the current study may be because two cultures were obtained from the children four weeks apart. This methodology is likely to produce a higher

| Exposure                                      | mecaA gene | cOR (95% C.I) | P-value |
|----------------------------------------------|------------|---------------|---------|
| Age (years)                                  |            |               |         |
| < 5                           | 1           | 3             | 0.73 (0.08\textasciitilde6.04) | 0.77  |
| ≥ 5                           | 8           | 175           | 1       |       |
| Sex                                          |            |               |         |
| Male                                        | 7           | 108           | 3.14 (0.64\textasciitilde15.50) | 0.14  |
| Female                                      | 2           | 97            | 1       |       |
| Doses of HAART\textsuperscript{a} missed in previous week\textsuperscript{b} | | |         |
| None                                        | 2           | 56            | 0.73 (0.15\textasciitilde3.62) | 0.70  |
| One or more                                 | 7           | 143           | 1       |       |
| On antibiotic prophylaxis (SMP-TMX/INH)\textsuperscript{c} | | |         |
| Yes                                         | 6           | 165           | 0.48 (0.12\textasciitilde2.02) | 0.31  |
| No                                          | 3           | 40            | 1       |       |
| Treatment with antibiotics in previous three months | | |         |
| Yes                                         | 5           | 29            | 7.58 (1.92\textasciitilde29.92) | <0.01 |
| No                                          | 4           | 17            | 1       |       |
| History of hospitalisation in the previous year | | |         |
| Yes                                         | 6           | 14            | 27.29 (6.16\textasciitilde120.87) | <0.01 |
| No                                          | 3           | 191           | 1       |       |
| Viral load (n = 200)\textsuperscript{d}     |            |               |         |
| Yes                                         | 2           | 102           | 0.25 (0.05\textasciitilde1.23) | 0.07  |
| No                                          | 7           | 89            | 1       |       |

\textsuperscript{a} HAART: Highly active antiretroviral therapy.
\textsuperscript{b} Six respondents not on HAART.
\textsuperscript{c} SMP-TMX/Isoniazid — Sulfamethoxazole trimethoprim/Isoniazid.
\textsuperscript{d} Viral load unavailable for 14 patients.
prevalence as both transient and persistent carriers of *S. aureus* were identified.

Using cefoxitin disc diffusion, the prevalence of MRSA carriage was 2.8%, while it was 4.2% using mecA gene detection. Although the prevalence of MRSA carriage was relatively low, the presence of the MRSA in the study population and the risk of autoinfection and transmission to others both within and outside the hospitals pose a serious public health threat. Among CLWH in Ghana, a similar nasal MRSA carriage of 3.4% was reported [14]. Among adults living with HIV in Lagos nasal MRSA carriage was 5.2% [24]. Nasal MRSA colonisation in the study was 2.8%, while it was 4.2% using cefoxitin disc diffusion [20]. Using cefoxitin disc diffusion, the prevalence of MRSA carriage was 5.2% [24]. Nasal MRSA colonisation in the CLWH in the present study corresponds to the colonisation rates of 9–17% reported by Hidron et al. among HIV-infected outpatients [25]. In South African CLWH, McNally et al. reported nasopharyngeal MRSA carriage in 29/239 (12.1%) of children [26]. The higher nasal MRSA carriage from the South African study may be because it was carried out in hospitalised CLWH unlike the present study that investigated outpatients. In CLWH in Ethiopia, MRSA colonisation rates were 16.8% [16]. The Ethiopian study investigated MRSA carriage in multiple sites including the skin and perineum unlike the present study in which only the anterior nares were swabbed. In the Botswana study where two nasal swabs were taken on separate occasions, MRSA carriage was 21% among CLWH [23].

This study suggested that hospitalisation within the previous year was an independent risk factor for MRSA colonisation. This finding is consistent with other reports about people living with HIV [25,27,28] and it may suggest that MRSA was hospital-acquired. Hospitals are known sources of MRSA and nasal colonisation with *S. aureus* is known to persist in a subset of individuals [29,30]. Although some of the respondents were hospitalised more than six months before the swabs were taken, the hospitals may still be the source of the organism. There was no association with household risk factors such as overcrowding, contact with animals, sharing personal items and MRSA colonisation identified in this study. This further suggests the likely hospital-acquired origin of MRSA.

A history of recent antibiotic therapy was also an independent risk factor for MRSA carriage. This is consistent with previous studies in populations living with HIV [27,31]. Cenizal et al. reported that the use of SMP-TMX was protective against MRSA colonisation [27]. In the present study, SMP-TMX prophylaxis did not have any protective effect against MRSA colonisation probably because >80% of *S. aureus* isolates were resistant to SMP-TMX. More than 75% of the participants in the current study were on SMP-TMX prophylaxis compared to 20% in the study by Cenizal et al. The exposure to SMP-TMX may have played role in the high rate of SMP-TMX resistance observed.

Due to the low number of MRSA isolates in the present study, the power to detect the association between MRSA colonisation and other factors such as viral suppression may have been too low. Although antibiotics are prescription drugs in Nigeria, they are still available without prescriptions from markets, street peddlers and patent medical stores [32]. As a result, the association of MRSA colonisation with recent antibiotic use may have been underestimated since antibiotics that were not prescribed in health facilities but nevertheless taken by the patients may not have been entirely captured. Even though this study involved only a single swab from the anterior nares and no other areas of the body, the few *S. aureus* isolates obtained have given an insight into the antimicrobial susceptibility of the organisms present in the population of CLWH and will be useful in guiding antimicrobial stewardship policy in the study sites.

### Conclusion

*S. aureus* nasal colonisation among CLWH attending clinics in Kano was comparable to that of similar populations in Africa. The antibiotic susceptibility profile of the isolates revealed that most *S. aureus* were meticillin-sensitive *S. aureus* (MSSA). Sensitivity to cefoxitin was high indicating that third-generation cephalosporins may still be appropriate empirical therapy for suspected staphylococcal infections in CLWH. Despite the low prevalence of MRSA among the study population, the potential spread of these resistant strains is concerning. We recommend that the Infection Prevention and Control units of the concerned hospitals should institute a combination of MRSA screening, decolonisation, and promotion of strict adherence to hand hygiene procedures to control the spread of MRSA. CLWH and their caregivers should also be educated to avoid sharing personal items and to adhere to hand hygiene procedures to minimise household spread.

### Authors Contributions

Aisha Sadauki, Abdulhakeem Olorukooba, Kabir Sabitu, Hye-Dalny Waziri, Mahmood Dalhat, Mukhtar Abdulaziz, Muhammad Balogun and Fatimah Hassan-Hanga: Conceptualization.

Aisha Sadauki: Data curation.
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Conflict of interest statement

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