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CASE REPORT

# Persistent *Staphylococcus aureus* nasal colonization in ambulatory human immunodeficiency virus-infected patients in Nigeria: Risk factors and molecular features

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This longitudinal study on *Staphylococcus aureus* colonization in Nigerian human immunodeficiency virus patients ($n = 187$) found a trend towards a higher proportion of persistent *S. aureus* carriage in patients with advanced human immunodeficiency virus infection, low CD4+ cell counts, and a predominance of isolates belonging to ST8/spa-CC064 in persistent carriers.


## Introduction

*Staphylococcus aureus* nasal colonization is an important risk factor for various *S. aureus* infections.[1] Persistent carriers and others (formerly: intermittent and noncarriers) are distinguished based on the risk of infection and anti-staphylococcal antibody pattern.[2]

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*S. aureus* nasal carriage is higher among human immunodeficiency virus (HIV) infected patients than in healthy individuals.[3] However, most investigations conducted in Africa analyzed a single nasal swab only and risk factors for persistent *S. aureus* nasal carriage was not considered. This study was undertaken to provide information on *S. aureus* persistent nasal carriage among HIV infected outpatients in Nigeria.

## Brief report

This longitudinal study comprised 187 ambulatory HIV infected patients, asymptomatic for any *S. aureus* infection attending the outpatient clinic of Mainland General Hospital, Lagos, Nigeria, between November 2008 and October 2009. Ethical approval was obtained from the Institutional Review Board of the Nigeria Institute of Medical Research, Lagos, Nigeria (IRB/08/065). Written informed consent was obtained from each participant prior to enrollment. Hospitalized patients were excluded from this study. Sociodemographic characteristics and medical history [duration of antiretroviral (ARV) therapy, cotrimoxazole prophylaxis, CD4+ cell count and stage of HIV infection as defined by the World Health Organization] were recorded in a standardized questionnaire. Two or three consecutive nasal swabs were collected randomly from each participant at an interval of 3 months. The swabs were cultured on 5% sheep blood agar (Oxoid, Basingstoke, Hampshire, UK) and mannitol salt agar (Oxoid) at 37°C overnight. Presumptive colonies were identified as *S. aureus* based on positive results for catalase, tube coagulase, and latex agglutination tests (Diamondial, Sees, France) and confirmed by polymerase chain reaction detection of the coagulase gene.[4] Susceptibility testing was performed by the Kirby–Bauer agar disc diffusion method using the following antibiotics: penicillin (10 units), methicillin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), erythromycin (15 μg), ciprofloxacin (5 μg), and cotrimoxazole (1.25/23.75 μg), and was evaluated according to Clinical Laboratory Standard Institute breakpoints. Persistent carriage was defined as isolation of *S. aureus* in at least two consecutive nasal swabs. Resistance to methicillin was confirmed by detection of *mecA*. Detection of the Panton–Valentine leukocidin gene (*luk*F-PV, *luk*S-PV) and *spa* typing (Staph Type 2.1.1; Ridom GmbH, Münster, Germany) was done as published.[5] Clusters of related *spa* types (spa-clonal complex, spa-CC) were identified using the based upon repeat pattern algorithm with preset parameters as published.[6] Multilocus sequence typing was carried out on the four most prevalent *spa* types.[7] Statistical analyses were performed using R and the package epicalc. A logistic regression analysis was used to compute the probability of persistent *S. aureus* colonization (outcome) under the studied set of risk factors (exposures) in a persistent carrier vs. others comparison. All risk factors with a significance level of $p < 0.1$ in the univariate analysis were adjusted for age and sex in a multivariate analysis. Age and sex are known risk factors for persistent carriage.[8] Association between categorical variables was assessed using Chi-square test and Fisher's exact test where appropriate and odds ratios (OR) and the 95% confidence interval (95% CI) was calculated. The significance level was 5%.

Women were predominant in the whole study population (80.2%, $n = 150$), and in persistent carriers only ($n = 40$, 78.4%). Out of the 187 patients included in this study, a total of 500 nasal swabs were collected. Of these, 176 (35%) patients were positive for *S. aureus*. Fifty-one (27%) HIV patients were defined as persistent carriers. Fifty-three (28%) patients were intermittent carriers and 83 (44%) were noncarriers. Low CD4+ cell counts were a strong risk factor of persistent carriage (Table 1). The proportion of persistent carriage was increased compared to nonpersistent colonization in patients with advanced HIV infection (WHO II/III, 21.6% vs. 11.8%, OR = 2. 1, 95% CI: 0.8–5.2, $p = 0.09$, Table 1) but we failed to detect statistical significance. The association of persistent carriage with low CD4+ cell counts and advanced HIV stage remained stable after adjusting for age and sex (Table 1). There was a lower proportion of persistent carriers in participants, who were

**Table 1** Risk factors for persistent *Staphylococcus aureus* nasal carriage in human immunodeficiency virus patients, Nigeria, 2008–2009

| | Predictors | Persistent carrier ($n = 51$) | Others (intermittent and non-carrier) ($n = 136$) | Crude OR (95%[b] CI) | $p$ | Adjusted OR[a] (95%[b] CI) | $p^a$ |
|---|---|---|---|---|---|---|---|
| Sex, female | *n* (%) | 40 (78.4) | 110 (80.9) | 0.9 (0.4–2.1) | 0.7 | | |
| Age (y) | Mean ± SD | 39.9 ± 9.9 | 41.0 ± 9.1 | 1.0 (1.0–1.0) | 0.5 | | |
| CD4+ cell count[b] (cells/μL) | Mean ± SD | 173.7 ± 106.8 | 484.4 ± 213.2 | 1.0 (1.0–1.0) | <0.001 | 1.0 (1.0–1.0) | <0.001 |
| HIV stage I | Number (%) | 40 (78.4) | 120 (88.2) | 0.5 (0.2–1.3) | 0.09 | 0.5 (0.2–1.1) | 0.1 |
| HIV stage II/III | Number (%) | 11 (21.6) | 16 (11.8) | 2.1 (0.8–5.2) | 0.09 | 2.1 (0.9–4.8) | 0.1 |
| Duration of current ARV (mo) | Median (range) | 6 (0-60) | 12 (0-60) | 1.0 (1.0–1.0) | 0.6 | | |
| Duration of current cotrimoxazole prophylaxis (mo) | median (range) | 7 (0-96) | 14 (0-60) | 1.0 (1.0–1.0) | 0.3 | | |

ARV = antiretroviral drug; CI = confidence interval; OR = odds ratio; SD = standard deviation.
[a] Adjusted for age, sex.
[b] CD4+ counts were not available for two persistent carriers and six others.

on ARV drugs for >1 year compared to participants who were on ARV for <1 year (13.7% vs. 25.7%, OR = 0. 5, 95% CI: 0.2–1.2, $p$ = 0. 08). The antimicrobial susceptibility tests of nonduplicates isolates showed high resistance rates to cotrimoxazole (92%, $n$ = 112), chloramphenicol (53%, $n$ = 65), methicillin (16%, $n$ = 20), ciprofloxacin (25%, $n$ = 31), erythromycin (65%, $n$ = 79), tetracycline (71%, $n$ = 86), and penicillin (100%, $n$ = 122). The *mecA* gene was detected in all phenotypically methicillin-resistant isolates. Notably, the mean number of CD4+ cells was significantly lower in methicillin-resistant *S. aureus* (MRSA) carriers vs. others, comprising methicillin-susceptible *S. aureus* carriers and noncarriers (293.7/μL vs. 413.4/μL, $p$ = 0.03).

The PVL gene was detected in 71 (40%) isolates. Of the 51 (100%) persistent carriers, 33 (64%) carried *S. aureus* with the same *spa* types, and 17 (33%) harbored isolates belonging to completely different *spa* types. The *spa* types of isolates from persistent carriers belonged to the following spa-CCs: spa-CC 064 [*spa* types (number of isolates in brackets): t008 (1), t064 (12), t304 (1), t967 (1), t1476 (1), t2658 (1), t6863 (2), t7808 (1)]; spa-CC 3772 [t3772 (12), t4683 (1), t6206 (1), t7802 (1)]; spa-CC 084 [t084 (4), t085 (1), t774 (1), t7806 (1)]; spa-CC 311 [t311 (3), t1400 (1), t7801 (1)]; spa-CC1156/7805 [t1156 (1), t7805 (1)]; spa-CC7796/7857 [t7796 (1)]; spa-CC7810/7856 [t7856 (1)]; spa-CC786 [t786 (1), t1603 (1)]; and spa-CC355/1172 [t355 (3)]. Isolates belonging to *spa* types t2304 (2), t2554 (3), t4976 (1), and t5058 (1) were excluded from this analysis (repeats <5). Also *spa* type t159 (1), t318 (1), t1458 (2), and t7813 (1) were singletons, and isolate from one persistent carrier was not typeable. The *spa* types from intermittent carrier clustered into spa-CC 064 [t064 (3), t197 (1), t701 (1), t1476 (1), t1774 (1)]; spa-CC 3772 [t3662 (1), t3772 (9), t4678 (2), t4683(1), t7807 (1), t7817 (2)]; spa-CC 084 [t084 (4)]; spa-CC 311 [t311 (3)]; spa-CC7796/7857 [t7857 (1)]; spa-CC7810/7856 [t7810 (1)]; spa-CC786 [t786 (2), t1603 (1), t4385 (1)]; and spa-CC355/1172 [t1172 (1)]. Isolates belonging to *spa* types t2304 (3) and t4976 were excluded from analysis (repeats <5), while *spa* type t934, t939 and t7804 were singleton, and nine isolates were not typeable. The following *spa* types and corresponding STs were most frequently found: t3772/ST25 ($n$ = 38), t084/ST15 ($n$ = 14), t064/ST8 ($n$ = 28), and t311/ST5 ($n$ = 7). Interestingly, spa-CC 064 was clearly associated with persistent carriage (OR = 2.7, 95% CI = 1.0–8.2, $p$ = 0.04).

## Discussion

This is the first study from Nigeria reporting the proportion of persistent *S. aureus* nasal carriage in HIV-patients and the associated risk factors for persistent *S. aureus* nasal carriage. The reason for the higher number of female patients visiting the HIV outpatient clinic might point toward a higher health awareness or burden of HIV infection in young women which is mirrored by a 3–7-fold higher prevalence of HIV infection among women compared to adolescent men in sub-Saharan Africa.[9] The persistent carriage of 27% in this study reflects the proportion of persistent carriers in Dutch HIV patients (29.6%) but is higher compared to healthy Dutch volunteers (24%).[10] We found that a low CD4+ cell count is significantly associated with persistent carriage (Table 1). It is known that the impairment of the immunosystem is a risk factor for *S. aureus* colonization in general and persistent colonization in particular. While the exact mechanism of *S. aureus* clearance remains largely unknown in humans, mouse models suggest that decolonization of *S. aureus* is T-cell dependent.[11]

Our results indicate that *S. aureus* of HIV-infected patients display a high rate of antimicrobial resistance; most of the isolates were resistant to three or more groups of antimicrobial agents. The reason is that antimicrobial resistance is common in Nigeria as there are no guidelines and restricted access to drugs.[12] The higher resistance rate against cotrimoxazole might be associated with the frequent use of this compound for *Pneumocystis jirovecii* prophylaxis, but no association between cotrimoxazole prophylaxis and cotrimoxazole resistance in *S. aureus* isolates was detected (OR = infinity, 95% CI: 0.23–infinity, $p$ = 0.6).

The proportion of PVL positive isolates in this study was high, which is similar to other reports from Africa.[5] In addition, not all *S. aureus* from persistent carriers showed the same genotype, indicating the diversity of strains colonizing the same patients. This information is important in planning intervention strategy necessary to prevent serious *S. aureus* infection in this population.

Two limitations of this study need to be addressed. First, we did not collect all relevant factors associated with colonization of *S. aureus*. Hence, caution should be applied in generalization of our findings. Second, we did not apply the cefoxitin screen test as recommended by Clinical Laboratory Standard Institute to screen for MRSA. As the methicillin/oxacillin disks are less sensitive in the detection of methicillin resistance, we might underestimate the prevalence of MRSA in our study. However, the MRSA phenotype is reliable, as all isolates were *mecA* positive.

In conclusion, the proportion of persistent carriers seems to increase with advanced immunosuppression, but can be reduced by long-term use of ARV therapy.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

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