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Staphylococcus aureus nasal colonization among HIV-infected adults in Botswana: prevalence and risk factors

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ABSTRACT

We sought to determine the clinical and epidemiologic determinants of *Staphylococcus aureus* nasal colonization in HIV-infected individuals at two outpatient centers in southern Botswana. Standard microbiologic techniques were used to identify *S. aureus* and methicillin-resistant *S. aureus* (MRSA). In a sample of 404 HIV-infected adults, prevalence of *S. aureus* nasal carriage was 36.9% ($n = 152$) and was associated with domestic overcrowding and lower CD4 cell count. MRSA prevalence was low ($n = 13$, 3.2%), but more common among individuals with asthma and eczema. The implications of these findings for HIV management are discussed.

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KEYWORDS
S. aureus; HIV; sub-Saharan Africa; nasal colonization

Introduction

Staphylococcus aureus is an important cause of morbidity and mortality in sub-Saharan Africa (SSA), especially among individuals infected with the human immunodeficiency virus (HIV) (Heysell, Shenoi, Catterick, Thomas, & Friedland, 2011; Taiwo, 2009). A major risk factor for staphylococcal infection is asymptomatic *S. aureus* colonization of the anterior nares.(Boyce, 1998; Herold et al., 1998; Ruimy et al., 2010). Understanding the epidemiology of *S. aureus* and MRSA colonization in Africa is important, as recent research indicates that since the year 2000, the prevalence in many African countries has increased and poses a threat to the continent (Falagas, Karageorgopoulos, Leptidis, & Korbila, 2013; Ghebremedhin et al., 2009; Schaumburg et al., 2011). Our objective was to define the prevalence and epidemiology of *S. aureus* nasal carriage among HIV-infected adults in Botswana.

Methods

We conducted this cross-sectional study at two clinical sites in southern Botswana: a large academic teaching hospital in the urban center of Gaborone (Princess Marina Hospital) and a rural district general hospital 36 km from Gaborone (Bamalete Lutheran Hospital). Study personnel at each site recruited participants to join the study while patients were waiting for routine clinic visits between March and June 2013. All adults were eligible to participate if they were receiving care at either study site and consented to participate in the study.

At enrollment, participants were interviewed regarding sociodemographic and clinical characteristics, including HIV treatment, and potential risk factors for *S. aureus* carriage. Laboratory parameters, including most recent CD4 cell count (cells/mm^3) and viral load (copies/mm^3) were obtained from medical records. One nasal sample was obtained from each participant at the time of enrollment and a second four weeks later.

Nasal samples were collected by rolling a sterile, unmoistened swabette (BBL™ CultureSwabs™ Liquid Stuart) in a 360 degree arc around the anterior nares. Each swab was streaked onto Manitol Salt Agar (MSA; Remel Inc.) and incubated at 37°C for 48 h to distinguish *S. aureus* from mixed flora. Each swab was then enriched in tryptic soy broth (Remel Inc.) for 24 h at 37°C and streaked a second time on MSA. Isolates presumptively identified as *S. aureus* on MSA that were also positive for coagulase were classified as *S. aureus* and further incubated on blood agar (Quad Five) at 37°C for 24 h to assess β-hemolysis and on tryptic soy agar (Remel Inc.) to test for catalase activity (Sigma). Sensitivity to methicillin was determined by Oxacillin E-test, and isolates were classified as MRSA at MIC ≥4 μg/ml and as methicillin-sensitive *S. aureus* (MSSA) at MIC

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≤2 μg/ml. Since participants were sampled on two separate occasions, *S. aureus* carriage was defined as either persistent or intermittent, based on the number of swabs from which *S. aureus* was cultured (2 or 1 swabs, respectively).

To assess the association between *S. aureus* colonization and potential risk factors, Poisson regression with robust variance estimator was used to compare prevalence of carriage to non-carriage in univariate and multivariate analysis and report prevalence ratios with 95% confidence intervals (reported as PR [95% CI], *p*-value) (Barros & Hirakata, 2003; Coutinho, Scazufca, & Menezes, 2008; Zou, 2004). All variables significant at $p < 0.20$ were considered for multivariate analysis and a final model retained those with $p < 0.05$ or that improved model fit. All analyses were conducted using Stata 14 (StataCorp LP, College Station, TX, USA).

This research was approved by the Health Research and Development Committee of the Botswana Ministry of Health and by institutional review boards at both study sites, The University of Texas Health Science Center, and the University of Pennsylvania.

Results

A total of 404 HIV-infected adults completed both visits; 72.8% ($n = 294$) were women. The median age was 43 years (range 21, 68). Most participants lived in rural settings ($n = 294$, 69.3%), and the average household size was 4 persons (range 1, 14). Almost all ($n = 396$, 98.0%) participants were on antiretroviral therapy (ART), and most ($n = 366$, 90.5%) had an undetectable viral load (<400 copies/mm^3). The overall prevalence of *S. aureus* nasal colonization was 36.9% ($n = 172$). Of those colonized, 48.7% ($n = 74$) were persistently colonized (Table 1).

In univariate analysis, carriage was more common in adults living in households with four or more individuals (Prevalence Ratio [PR] 1.46 [CI]: 1.13, 1.90], $p = 0.004$) and in households where children resided (PR 1.30 [95% CI: 1.002, 1.68). Those living in rural areas were also more likely to be colonized than urban dwellers (PR 1.72 [95% CI: 1.14, 2.60], $p = 0.010$). Carriage was more common in individuals with a CD4 count <750 cells/mm^3 (PR 1.58 [95% CI: 1.01, 2.46], $p = 0.045$) and among those individuals with co-morbid asthma (PR 1.52 [95% CI: 1.07, 2.14], $p = 0.019$). History of prior hospitalization and current use of antibiotics were also associated with carriage (PR 1.36 [95% CI: 1.05, 1.75], $p = 0.019$, and PR 1.79 [95% CI: 1.003, 3.20], $p = 0.049$, respectively).

In multivariate analysis, having a CD4 count <750 cells/mm^3 was associated with an 80% increase in the prevalence of *S. aureus* carriage, and carriage was more common in those living longer with HIV (PR 1.80 [95% CI: 1.12, 2.90], $p = 0.015$ and PR 1.04 [95% CI:.1.00, 1.08] $p = 0.036$ respectively). Being hospitalized in the previous seven years and living in households with children were associated with a 36% and 45% increased prevalence, respectively (PR 1.36 [95% CI: 1.03, 1.79], $p = 0.029$ and PR 1.45 [95% CI:.1.09, 1.92] $p = 0.0011$ respectively) (Table 2).

MRSA carriers accounted for 8.5% ($n = 13$) of all *S. aureus* carriers, for an overall MRSA prevalence of 3.2%. In multivariate analysis, having CD4 count <350 cells/mm^3 was more common in those colonized with MRSA compared to MSSA (PR 5.28 [1.35, 20.66), $p = 0.017$). History of asthma (PR 9.43 [1.13, 78.56], $p = 0.038$) and eczema (PR 5.23 [1.14, 23.97], $p = 0.033$) were also more likely in those individuals colonized with MRSA.

Discussion

The prevalence of *S. aureus* nasal carriage in this large population of HIV-infected adults in Botswana was 37%. While this was similar to what has been described in other highly HIV prevalent settings, (Akoua Koffi et al., 2004; Gebreyesus, Gebre-Selassie, & Mihert, 2013; Omuse, Kariuki, & Revathi, 2012) prevalence of MRSA colonization was lower than reported elsewhere in SSA (Heysell et al., 2011).

Although there were very few individuals with advanced HIV/AIDS in this study population, those with CD4 counts <750 cells/mm^3 were more likely to be colonized with *S. aureus*. That carriage was not predicted by a CD4 threshold lower than 750 cells/mm^3 could be due to the small number of individuals with advanced immunosuppression in our analysis, rather than a lack of association with more advanced disease. Duration of HIV-infection was associated with carriage, but HIV viremia was not. While there is substantial existing research demonstrating that HIV infection increases vulnerability(Groome, Albrich, Wadula, Khoosal, & Madhi, 2012; Miller et al., 2007; Zervou, Zacharioudakis, Ziakas, Rich, & Mylonakis, 2014), the lack of association between viral load and colonization in our analysis supports a growing body of evidence showing that virologic control on HAART is protective against *S. aureus* colonization (Cenizal, Hardy, Anderson, Katz, & Skiest, 2008; Cotton, Wasserman, Smit, Whitelaw, & Zar, 2008; Hidron, Kempker, Moanna, & Rimland, 2010; Miller et al., 2007).

As expected, *S. aureus* carriage was associated with higher household density and living with children. These findings are consistent with research from high

Table 1. Factors associated with *S. aureus* nasal carriage in HIV-infected participants, unadjusted ($N = 404$).

	Non-carriage *n* (%)	Intermittent carriage *n* (%)	Persistent carriage *n* (%)		*p*-value
	252 (62.38%)	78 (19.00%)	74 (18.00%)	Carriage vs. non-carriage PR (95% CI)	
Demographic Factors					
Age (years)‡ [median (IQR)]	43.3 (21, 68)	44.4 (29, 66)	41.2 (21, 60)	0.99 (0.98, 1.01)	0.623
Sex					
Male	73 (66.36%)	20 (18.18%)	17 (15.45%)	1.00 (ref)	
Female	179 (60.88%)	58 (19.73%)	57 (19.39%)	1.16 (0.86, 1.57)	0.323
Residence					
Urban	87 (70.16%)	17 (13.71%)	20 (16.13%)	1.00 (ref)	
Semi-rural	18 (48.65%)	7 (18.92%)	12 (32.43%)	1.72 (1.14, 2.60)	
Other	146 (60.33%)	54 (22.31%)	42 (17.36%)	1.33 (0.97, 1.82)	0.010
Household Size (no. of persons)‡ [median (range)]	3.8 (1, 14)	4.4 (1, 12)	4.2 (1, 13)	1.05 (1.01, 1.10)	0.027
Household Size ≥4					
No	139 (69.50%)	29 (14.50%)	32 (16%)	1.00 (ref)	
Yes	113 (55.39%)	49 (24.02%)	42 (20.59%)	1.46 (1.13, 1.90)	0.004
Children <13 years in Household					
No	131 (67.53%)	31 (15.98%)	32 (16.49%)	1.00 (ref)	
Yes	121 (57.89%)	46 (22.01%)	42 (20.1%)	1.30 (1.002, 1.68)	0.049
Health History					
Eczema					
No	230 (63.54%)	71 (19.61%)	61 (16.85%)	1.00 (ref)	
Yes	21 (52.50%)	7 (17.50%)	12 (30.00%)	1.30 (0.91, 1.85)	0.143
Asthma					
No	238 (63.81%)	69 (18.5%)	66 (17.69%)	1.00 (ref)	
Yes	14 (45.16%)	9 (29.03%)	8 (25.81%)	1.52 (1.07, 2.14)	0.019
Has Ever had Tuberculosis?					
No	165 (62.03%)	45 (16.92%)	56 (21.05%)	1.00 (ref)	
Yes	87 (63.04%)	33 (23.91%)	18 (13.04%)	0.97 (0.75, 1.27)	0.843
Hospitalized in Past 7 Years?					
No	174 (67.18%)	45 (17.37%)	40 (15.44%)	1.00 (ref)	
Yes	76 (55.47%)	30 (21.9%)	31 (22.63%)	1.36 (1.05, 1.75)	0.019
Lives >4 km from Routine Healthcare					
No	143 (60.08%)	53 (22.27%)	42 (17.65%)	1.00 (ref)	
Yes	105 (65.22%)	24 (14.91%)	32 (19.88%)	0.87 (0.67, 1.13)	0.305
Currently on Antibiotics?					
No	250 (62.81%)	75 (18.84%)	73 (18.34%)	1.00 (ref)	
Yes	2 (33.33%)	3 (50.00%)	1 (16.67%)	1.79 (1.003, 3.20)	0.049*
Shares Bar Soap with Others					
No	91 (69.47%)	19 (14.50%)	21 (16.03%)	1.00 (ref)	
Yes	161 (58.97%)	59 (21.61%)	53 (19.41%)	1.34 (1.0001, 1.81)	0.050
HIV-Related Factors					
Time since HIV Diagnosis (years)‡ [median (IQR)]	8.1 (0, 19.0)	9.1 (0.6, 23)	8.3 (0.1, 19.7)	1.03 (0.99, 1.06)	0.117
Viral Load (copies/mm^3)					
Undetectable (<400)	230 (62.84%)	73 (19.95%)	63 (17.21%)	1.00 (ref)	
≥400	19 (61.29%)	3 (9.68%)	9 (29.03%)	1.04 (0.66, 1.66)	0.863
CD4 Cell Count (cells/mm^3)					
≥750	48 (75.00%)	9 (14.06%)	7 (10.94%)	1.00 (ref)	
<750	203 (60.60%)	67 (20.00%)	65 (19.40%)	1.58 (1.01, 2.46)	0.045

IQR – Inter Quartile Range.
*Contains an association with frequency ≤5.
‡Continuous variable: PR should be interpreted as the per-unit increase in prevalence.

income settings, where overcrowding and poverty have been found to correlate strongly with higher *S. aureus* carriage (Gorwitz et al., 2008; Leung, Padgett, Robinson, & Brown, 2015). Whether colonization risk associated with these factors is related to unhygienic living conditions or related to transmission within social networks in overcrowded domestic settings (Miller & Neaigus, 2001) is unclear and warrants further analysis.

The prevalence of MRSA was lower than previously reported in other high HIV prevalent settings in southern Africa (Cotton et al., 2008; Groome et al., 2012; Heysell et al., 2011) and lower than was reported in a recent multi-country meta-analysis that found 6.9% of all HIV-infected individuals, across a variety of settings, carried MRSA (Zervou et al., 2014). MRSA was associated with asthma and eczema. It is unclear whether this reflects more frequent access to healthcare services in patients with asthma and eczema, increasing risk for nosocomial MRSA acquisition, or disease-induced modification of the nares, conducive to MRSA carriage, specifically.

To our knowledge, this is the first study to employ a two-swab method to characterize *S. aureus* carriage, distinguishing persistent from intermittent carriers, in a

Table 2. Factors independently associated with *S. aureus* nasal carriage in HIV-infected participants, age- and sex-adjusted (*N* = 404).

Factors	Carriage vs. non-carriage PR (95% CI)*	*p*-value
Time since HIV Diagnosis (years)‡	1.04 (1.00, 1.08)	0.036
CD4 cell count (cells/mm^3)		
≥750	1.00 (ref)	
<750	1.80 (1.12, 2.90)	0.015
Viral load (copies/mm^3)		
Undetectable (<400)	1.00 (ref)	
≥400	1.00 (0.82, 1.23)	0.968
Household size (no. of persons)		
<4	1.00 (ref)	
≥4, without children <13 years old	1.38 (0.86, 2.22)	0.186
≥4, with children <13 years old	1.45 (1.09, 1.92)	0.011
Hospitalized in Past 7 Years?		
No	1.00 (ref)	
Yes	1.36 (1.03, 1.79)	0.029
Asthma		
No	1.00 (ref)	
Yes	1.48 (0.97, 2.24)	0.067

**Adjusted for age and sex.
‡Continuous variable: PR should be interpreted as the per-unit increase in prevalence.

highly prevalent HIV region and the largest study of its kind in an HIV outpatient setting in SSA. A major strength of our study is the assessment of carriage at two distinct points in time, reducing the systematic misclassification of some intermittent carriers as non-carriers, an inherent bias in a single-swab approach. Persistent *S. aureus* nasal carriage occurred in the population with approximately the same frequency as intermittent carriage (18% and 19%, respectively). Had we relied on the first swab alone, only 117 (29.0%) of 404 participants would have been classified as carriers, while an additional 32 carriers (7.9% of participants), who carried *S. aureus* only intermittently, were classified as such only with a second culture.

A limitation of our study is the cross-sectional design, since definitive conclusions on causality cannot be made. Furthermore, we cannot exclude recruitment bias, since all participants were recruited from clinical settings and may not be representative of the wider community. The study also lacked power to characterize the epidemiology of *S. aureus* carriage in HIV-infected individuals who were not on treatment. Nevertheless, given that Botswana has achieved universal access to HAART (NACA, 2013), we believe that our results are valid for other HIV-infected adults on ART in Botswana.

Conclusion

In conclusion, there was a high prevalence of *S. aureus* nasal colonization in this HIV-infected adult population accessing care at HIV clinics in southern Botswana. However, prevalence of MRSA nasal carriage in this setting was low. Colonization was associated with domestic overcrowding, recent hospitalization, lower CD4 counts, and longer time since HIV diagnosis. MRSA carriage was associated with low CD4 counts, asthma, eczema, and rural living.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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