Prevalence of *Staphylococcus aureus* Nasal Carriage in Human Immunodeficiency Virus–Infected and Uninfected Children in Botswana: Prevalence and Risk Factors

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Abstract. *Staphylococcus aureus* is an important cause of morbidity and mortality in children in sub-Saharan Africa (SSA). A major risk factor for staphylococcal infection is *S. aureus* colonization of the anterior nares. We sought to define risk factors for *S. aureus* carriage and characterize antimicrobial resistance patterns in children in Botswana. A cross-sectional study was conducted at two clinical sites in southern Botswana. Patients under 18 years of age underwent two nasal swabs and brief interviews, 4 weeks apart. Standard microbiological techniques were used. For persistent carriers, *S. aureus* was isolated from swabs at both time points, and for intermittent carriers, *S. aureus* was isolated from only one swab. Poisson regression with robust variance estimator was used to compare prevalence of carriage and the resistance phenotypes. Among 56 enrollees, prevalence of *S. aureus* colonization was 55% ($N = 31$), of whom 42% ($N = 13$) were persistent carriers. Of human immunodeficiency virus–infected children, 64% ($N = 9$) were carriers. Risk factors for nasal carriage included a history of tuberculosis (prevalence ratio [PR] = 1.60; 95% confidence interval [CI] = 1.02, 2.51; $P = 0.040$) and closer proximity to health care (PR = 0.89; 95% CI = 0.80, 0.99; $P = 0.048$). Prior pneumonia was more common among persistent rather than intermittent carriers (PR = 2.64; 95% CI = 1.64, 4.23; $P < 0.001$). Methicillin-resistant *S. aureus* (MRSA) prevalence was 13%. Of isolates tested, 16% were resistant to three or more drugs ($N = 7/44$). In summary, children in southern Botswana are frequently colonized with *S. aureus*. Antibiotic resistance, especially MRSA, is also widespread. Antibiotic recommendations for treatment of staphylococcal infections in SSA should take cognizance of these resistance patterns.

INTRODUCTION

Staphylococcus aureus is one of the most common causes of pediatric infections worldwide, manifesting as a range of diseases, from minor skin infection to severe and fatal invasive disease.¹ In sub-Saharan Africa (SSA), *S. aureus* is a leading cause of skin and soft tissue infections,² as well as pneumonia and bacteremia.^{3–6} Nevertheless, despite evidence that children, especially immunocompromised children, are disproportionately vulnerable to staphylococcal infections,^{3,4,7–9} very little is known about the epidemiology of *S. aureus* in children in high human immunodeficiency virus (HIV)–prevalent settings in SSA. A greater understanding of the ecology of *S. aureus* in SSA is urgently needed, since effective prevention and judicious treatment strategies are critical in settings where clinical resources and antibiotic options are limited.^{10,11}

Nasal carriage of *S. aureus* is a major risk factor for staphylococcal infection, and *S. aureus* carriers are more prone to staphylococcal infection and experience recurrence of disease, especially if they are colonized persistently, compared with individuals who do not carry *S. aureus*.^{5,12} HIV infection has also been associated with a greater risk of colonization^{13,14} as well as infection; thus, children with HIV represent a high-risk group. We sought to describe the prevalence of *S. aureus* nasal colonization in children in southern Botswana. Botswana has been disproportionately affected by the HIV epidemic: an estimated 17,396 children (0–14 years

of age) were living with HIV in 2013 and 2.3% of children between 0 and 4 years of age are HIV infected.¹⁵ On account of recent evidence of an increase in methicillin-resistant *S. aureus* (MRSA) infections in Botswana,¹² we also described the prevalence of MRSA and characterized the antibiotic susceptibility phenotypes of the *S. aureus* nasal carriage isolates recovered.

METHODS

Data and sample collection. From March 26, 2013 to June 13, 2013, children 0–18 years of age attending well care in outpatient departments of Princess Marina Hospital (an urban 550-bed public hospital in Gaborone) and Bamalete Lutheran Hospital (a semiurban/rural 130-bed district hospital in Ramotswa) were invited to participate in this cross-sectional study to detect *S. aureus* in the nares and establish carriage status. Study personnel at each site directly recruited participants to join the study while patients were attending routine clinic visits. Children were eligible for recruitment if they were Botswana residents and receiving care at either study site. After signed parental consent and child assent in those ≥ 7 years of age, participants and their parents were interviewed by study personnel using a standardized questionnaire that addressed personal and demographic factors, health history, medication use, hygiene, and, where appropriate, HIV-related factors. HIV status was ascertained by parental interview, and HIV clinical data (viral load and CD4 cell count) were abstracted from the clinic medical record.

Bacterial specimens were collected by rolling a sterile, unmoistened swabette (BBL™ CultureSwabs™ Liquid Stuart, Becton, Dickinson and Company, Franklin Lakes, NJ) in a circular motion around the inside of the nares,¹⁶ and the swabs

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were transported within 8 hours to the Botswana National Health Laboratory in Gaborone for processing. Participants were asked to return after 4 weeks for a second nasal swab and brief interview to ascertain changes in health status.

Microbiological testing. The presence of *S. aureus* was determined by standard microbiological techniques.^{16,17} Each swab was streaked onto mannitol salt agar (MSA) (Remel Inc., Lenexa, KA) and incubated at 37°C for 48 hours. Swabs were incubated at 37°C for 24 hours in tryptic soy broth (Remel Inc.) and streaked a second time onto MSA.¹⁸ Colonies from all MSA cultures resembling *S. aureus* were subsequently inoculated into tryptic soy agar (TSA) and transported to the University of Texas School of Public Health in Houston for additional testing. In Houston, cultures were regrown on MSA and then incubated at 37°C for 24 hours on blood agar, to assess hemolysis and test for coagulase activity (BactiStaph Latex 450; Remel Inc.), and on TSA, to test for catalase activity (Sigma, St. Louis, MO). Isolates that were positive by these tests were considered to be *S. aureus*.

Finally, isolates were transferred to Mueller–Hinton agar for antibiotic susceptibility testing. Sensitivity to methicillin was detected using the Oxacillin E-test strip, and isolates were classified as MRSA at minimum inhibitory concentration (MIC) ≥ 4 $\mu\text{g/mL}$; this was performed on all *S. aureus* isolates. On a subset of *S. aureus* isolates, sensitivities to an additional 18 antibiotics were ascertained by the Kirby–Bauer disk diffusion method, using clinical breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), when available, and the Clinical Laboratory Standards Institute (CLSI).^{19,20} Antibiotic susceptibility data are presented as frequency of nonsusceptibility; nonsusceptible isolates were defined as those resistant by EUCAST/CLSI standards or with intermediate susceptibility (i.e., intermediate susceptibility is grouped with resistance in this report).

Assignment of carriage status. All participants completing two study visits were classified as either carriers or noncarriers of *S. aureus*. Carriers were individuals from whom *S. aureus* was cultured during either visit, and noncarriers were individuals from whom no *S. aureus* was cultured during both study visits. The subset of participants who were designated as *S. aureus* carriers were then further characterized by carriage type (persistent or intermittent), according to the standard “2 culture rule,” which validates that a minimum of 2 weeks is sufficient time to distinguish most persistent carriers from intermittent carriers.²¹ Persistent carriers were defined as individuals from whom *S. aureus* was isolated from swabs at both time points, and intermittent carriers were individuals from whom *S. aureus* was isolated from one swab, but not both.

Statistical analysis. Chi-square and Fisher's exact tests were used for univariate comparison between carriers and noncarriers to compare categorical variables (e.g., gender, staphylococcal infection history). Two-tailed unpaired Student's *t* test was used to compare the means of continuous variables (e.g., age, household size) between carriers and noncarriers. Poisson regression with robust variance estimator was used to obtain univariate prevalence ratios (PRs) and 95% confidence intervals (CIs), comparing carriers to noncarriers, by HIV status and other potential risk factors.^{22–24} To further describe factors associated with specific carriage

phenotypes, we also compared intermittent carriers to noncarriers and persistent carriers to noncarriers, using the same regression procedures.

Ethics. This study was reviewed and approved by the institutional review boards of the Botswana Ministry of Health, Princess Marina and Bamalete Lutheran Hospitals, The University of Texas Health Science Center, and the University of Pennsylvania.

RESULTS

Sixty children enrolled in the study, of which 93% (*N* = 56) completed both study visits and nasal swabs. Of the 56 that completed both visits, 29 (51.8%) were female and 14 (25%) were HIV infected. The mean age for HIV-infected study participants was significantly higher than HIV-uninfected individuals (11.5 years versus 3.9 years, *P* < 0.001) (Table 1). HIV-infected children were more likely to report previously having tuberculosis (TB), pneumonia, skin boils, or abscesses. All HIV-infected children were on antiretroviral therapy and most had undetectable viral counts (*N* = 12, 86%), as defined by national guidelines (<400 copies/mm³) (Table 2). Half of those in whom a recent CD4 T-cell count had been measured, had CD4 cell count percent greater than 25% (*N* = 5). Twelve HIV-uninfected individuals were born to HIV-infected mothers; all of these HIV-exposed children were ≥ 3 years of age.

***Staphylococcus aureus* nasal carriage.** Nasal carriage of *S. aureus* was detected in 55% of children that completed

TABLE 1
Characteristics of study population (*N* = 56), stratified by HIV status

	HIV status		<i>P</i> value
	HIV uninfected (<i>N</i> = 42) Mean $\pm$ SD or <i>n</i> (%)	HIV infected (<i>N</i> = 14) Mean $\pm$ SD or <i>n</i> (%)	
Demographic characteristics			
Age (years), mean $\pm$ SD	3.89 $\pm$ 4.45	11.53 $\pm$ 3.74	< 0.001**
Age (years)			
≤ 1	15 (35.7)	0 (0)	< 0.001*
2–3	14 (33.3)	1 (7.1)	
4–11	8 (19.0)	4 (28.6)	
12–16	5 (11.9)	9 (66.3)	
Sex			
Female	21 (50.0)	8 (57.1)	0.456*
Residence			
Urban	4 (9.5)	1 (7.2)	0.034*
Rural	15 (35.7)	10 (71.4)	
Other	23 (54.8)	3 (21.4)	
Health-related characteristics			
History of tuberculosis	1 (2.4)	5 (35.7)	0.003*
History of pneumonia	1 (2.4)	3 (21.4)	0.045*
History of skin boils or abscesses	1 (2.4)	8 (57.1)	0.004*
Health-care utilization			
Has ever been hospitalized	9 (21.4)	11 (78.6)	< 0.001*
Currently on antibiotics	1 (2.4)	2 (14.3)	0.151*
Use of TMP–SXT within past 6 months	8 (19.0)	3 (21.4)	1.000*

HIV = human immunodeficiency virus; SD = standard deviation; TMP–SXT = trimethoprim-sulfamethoxazole. Frequencies may not sum to total *N* because of missing values.

*Fisher's exact test.

**Student's *t* test.

TABLE 2
Characteristics of HIV-infected study participants (*N* = 14)

	Number (%)
Viral load (copies/mm ³)	
Undetectable (≤ 399)	12 (85.7)
> 399	2 (14.3)
CD4 cell count (%)*	
> 25%	5 (50.0)
$\leq 25\%$	5 (50.0)
Line of ARV therapy*	
1st	10 (83.3)
2nd	1 (8.3)
Custom	1 (8.3)
Currently on TMP-SXT for chemoprophylaxis	1 (7.1)

ARV = antiretroviral; HIV = human immunodeficiency virus; TMP-SXT = trimethoprim-sulfamethoxazole.

*Frequencies may not sum to total *N* because of missing values.

both study visits ($N = 31$) (Table 3). There was not a significant difference in overall carriage prevalence between HIV-infected children (64% [$N = 9$]) and HIV-uninfected children (52% [$N = 22$]; $PR = 1.23$; 95% $CI = 0.7, 2.0$; $P = 0.412$). Carriage was less frequent among children who lived farther than 4 km from the health clinics ($PR = 0.89$; 95% $CI = 0.8, 0.99$; $P = 0.048$), compared with those who lived closer than 4 km. Personal hygiene habits and sharing of hygiene items did not influence colonization, but individuals who shared clothes or other items for sports activities had a 2-fold higher prevalence ($PR = 2.00$; 95% $CI = 1.48, 2.70$; $P < 0.001$). Children with a history of TB were 60% more likely to be carriers ($PR = 1.60$; 95% $CI = 1.02, 2.51$; $P = 0.040$).

Persistence of *S. aureus* nasal carriage. Persistent carriage was detected in 23% ($N = 13$), and intermittent carriage in 32% ($N = 18$) of participants (Table 3). Prevalence of persistent carriage increased by 10% for every year of life ($PR = 1.10$; 95% $CI = 1.02, 1.20$; $P = 0.015$). Those attending school or preschool ($PR = 2.78$; 95% $CI = 1.02, 7.57$; $P = 0.046$), and children who shared sports items ($PR = 4.14$; 95% $CI = 2.15, 7.98$; $P < 0.001$) were more likely to be persistent carriers than any other carriage type. A prior history of pneumonia was also more common among persistent rather than intermittent carriers ($PR = 2.64$; 95% $CI = 1.64, 4.23$; $P < 0.001$).

Among those children living with HIV, those with uncontrolled viremia and recent trimethoprim-sulfamethoxazole (TMP-SXT) exposure were significantly more likely to be carriers of *S. aureus* than noncarriers (PR = 1.71; 95% CI = 1.04, 2.82; $P = 0.033$, and PR = 1.83; 1.05, 3.21; $P = 0.034$, respectively) (Table 4). HIV-infected children with uncontrolled viremia, recent TMP-SXT exposure, and/or a history of TB were more likely to be persistent carriers compared with intermittent carriers (PR = 2.25; 95% CI = 1.05, 4.84; $P = 0.038$, and PR = 2.25; 95% CI = 1.05, 4.84; $P = 0.038$ and PR = 2.30; 1.21, 4.38; $P = 0.011$, respectively). A history of skin abscesses or boils was not predictive of carriage status in HIV-infected children (data not included).

Antibiotic resistance of isolates. Thirteen percent ($N = 9$) of all the *S. aureus* isolates tested were MRSA by Oxacillin E-test. A further 10% ($N = 7$) of isolates exhibited intermediate susceptibility (borderline oxacillin-resistant *S. aureus* [BORSA], MIC = 3 $\mu\text{g/mL}$). The prevalence of MRSA carriage was 21% ($N = 3$) among HIV-infected children and 14% ($N = 6$) among HIV-uninfected children ($P = 0.76$). MRSA carriers

TABLE 3
Factors associated with *Staphylococcus aureus* nasal carriage

	Patients associated with <i>Campylobacter</i> serotype 104000000						Carriage status			
	Noncarriage (N = 25) Mean $\pm$ SD or n (%)	Intermittent carriage (N = 18) Mean $\pm$ SD or n (%)	Persistent carriage (N = 13) Mean $\pm$ SD or n (%)	PR: carriage vs. noncarriage (95% CI)*	P value	PR: intermittent carriage vs. noncarriage (95% CI)*	P value	PR: persistent carriage vs. noncarriage (95% CI)*	P value	
Demographic characteristics										
Mean age (years)	4.6 $\pm$ 4.9	5.0 $\pm$ 5.4	8.8 $\pm$ 5.6	1.0 (0.9, 1.1)	0.156	1.0 (0.94, 1.08)	0.817	1.09 (1.01, 1.18)	0.019	
Sex (female)	13 (48.15%)	9 (33.33%)	5 (18.52%)	0.88 (0.55, 1.43)	0.616	0.95 (0.47, 1.95)	0.898	0.69 (0.27, 1.76)	0.442	
Attends school or preschool	8 (33.3%)	7 (29.2%)	9 (37.5%)	1.42 (0.89, 2.27)	0.141	1.19 (0.58, 2.44)	0.639	2.78 (1.02, 7.57)	0.046	
Ever shares sports/exercise apparel	0	1 (20%)	4 (80%)	2.00 (1.48, 2.70)	< 0.001	2.47 (1.66, 3.66)	< 0.001	4.14 (2.15, 7.98)	< 0.001	
Distance to routine health care (km)	4.5 $\pm$ 3.4	2.1 $\pm$ 1.6	3.3 $\pm$ 3.5	0.89 (0.80, 0.99)	0.048	0.80 (0.7, 0.92)	0.002	0.92 (0.77, 1.10)	0.374	
Children < 5 years in household	20 (42.6%)	16 (34%)	11 (23.4%)	0.75 (0.45, 1.22)	0.245	1.05 (0.33, 3.32)	0.930	0.43 (0.19, 0.96)	0.040	
Health-related characteristics										
Has ever had pneumonia	2 (50%)	0	2 (50%)	1.55 (0.51, 4.68)	0.442	Undefined	–	2.64 (1.64, 4.23)	< 0.001	
Has ever had tuberculosis	1 (16.7%)	3 (50%)	2 (33.3%)	1.60 (1.02, 2.51)	0.040	1.95 (0.97, 3.92)	0.061	2.12 (0.82, 5.49)	0.121	
HIV infected	5 (35.7%)	3 (21.4%)	6 (42.9%)	1.23 (0.75, 2)	0.412	0.88 (0.33, 2.34)	0.790	2.10 (0.90, 4.9)	0.085	
Currently on antibiotics	1 (33.3%)	1 (33.3%)	1 (33.3%)	1.22 (0.52, 2.83)	0.647	1.21 (0.28, 5.14)	0.800	1.50 (0.34, 6.59)	0.591	

CI = confidence interval; HIV = human immunodeficiency virus; PR = prevalence ratio; SD = standard deviation. Frequencies may not sum to total *N* because of missing values.

Continuous variable: PR should be interpreted as the per-unit increase in prevalence.

TABLE 4
Factors associated with *Staphylococcus aureus* nasal carriage in HIV-infected children

	Carriage status					
	Noncarriage (N = 5) n (%)	Intermittent carriage (N = 3) n (%)	Persistent carriage (N = 6) n (%)	PR: carriage vs. noncarriage (95% CI)*	P value	PR: intermittent carriage vs. noncarriage (95% CI)*
Detectable viral load (> 400 copies/mm ³)	0	0	2 (100%)	1.71 (1.04, 2.82)	0.033	Undefined
CD4 cell count % < 25%	0	2 (40%)	3 (60%)	4 (0.66, 24.21)	0.131	Undefined
On first line ARV regimen	4 (36.4%)	2 (18.2%)	5 (45.5%)	0.95 (0.37, 2.47)	0.924	0.67 (0.10, 4.51)
TMP-SXT use in last 6 months	0	1 (33.3%)	2 (66.7%)	1.83 (1.05, 3.21)	0.034	3.50 (1.001, 12.24)
						2.25 (1.05, 4.84)
						2.25 (1.05, 4.84)
						0.038
						0.138
						0.896
						0.038

ARV = antiretroviral; CI = confidence interval; HIV = human immunodeficiency virus; PR = prevalence ratio; TMP-SXT = trimethoprim-sulfamethoxazole. Frequencies may not sum to total N because of missing values.
*Continuous variable: PR should be interpreted as the per-unit increase in prevalence.

accounted for 38% (N = 5) of persistent and 22% (N = 4) of intermittent carriers. No persistent carrier had MRSA on both swabs.

Susceptibilities to a broad panel of antibiotics was determined for a subset of 44 *S. aureus* isolates from 29 carriers (Table 5). Sixteen percent of these isolates (N = 7) showed resistance to three or more classes of drugs; they were isolated exclusively from intermittent carriers. Only two isolates demonstrated resistance to TMP-SXT and both were methicillin susceptible and came from HIV-uninfected children.

DISCUSSION

This first report on the epidemiology of *S. aureus* in a community-based population of children in Botswana demonstrates a high prevalence of *S. aureus*, especially MRSA, nasal carriage. Strikingly, 55% of participants were colonized by *S. aureus*, of which 29% harbored MRSA. We identified a high proportion of *S. aureus* isolates that were resistant to three or more antibiotics, underscoring that antibiotic resistance of *S. aureus* circulating in pediatric communities of SSA requires urgent attention.

***Staphylococcus aureus* in HIV-infected patients.** *Staphylococcus aureus* nasal colonization was not predicted by HIV status. Nevertheless, the prevalence in HIV-infected children was double than what has been reported among HIV-infected children in neighboring South Africa (24–36%)^{6,25,26} and higher than has been reported elsewhere in the world (24–45%).^{27,28} Consistent with established data, uncontrolled HIV viremia was a risk factor for colonization.²⁹ However, neither absolute CD4 cell count nor CD4% were predictive of carriage. Despite higher prevalence of *S. aureus* carriage among HIV-infected children with a history of boils or skin abscesses, skin infections were not predictive of carriage status in regression analysis. This lack of association between colonization and immunosuppression or skin infections may be because the study was underpowered, given the small number of patients in these subgroups.³⁰

Antibiotic-resistant *S. aureus*. The high prevalence and antibiotic resistance phenotype of BORSA isolates may reflect distinct and different mechanisms of methicillin resistance in *S. aureus* isolates in Botswana compared with other settings, such as hyperproduction of β -lactamases, rather than mediated by the penicillin-binding protein 2a that is encoded in *mecA* gene.^{31,32} Typically BORSA isolates are not resistant to multiple agents³³ and are believed to have little relevance.^{34,35} However, in our analysis, BORSA isolates demonstrated several resistance patterns. Further molecular analysis is necessary to better understand the epidemiology of this resistance pattern and to determine whether these isolates are responsible for substantial morbidity in southern Africa.

We did not demonstrate substantial TMP-SXT resistance, although a few children in the study reported recent exposure. Nevertheless, this finding is reassuring given widespread use of TMP-SXT, especially among HIV-infected children, in Botswana³⁶ and since high rates of TMP-SXT resistance have been reported elsewhere in SSA.^{37,38} Low levels of TMP/SXT resistance is consistent with data from high-income countries demonstrating that community-acquired strains of MRSA, containing the staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, are more

TABLE 5
Antibiotic resistance profile (*N* = 44 isolates)*

				Host characteristic				
				<i>n</i>	HIV negative (<i>N</i> = 29)	HIV infected (<i>N</i> = 14)	Persistent carrier (<i>N</i> = 27)	Intermittent carrier (<i>N</i> = 17)
MSSA								
Susceptible to all				2	1 (3.4%)	0	1 (3.7%)	1 (5.9%)
PEN				22	11 (37.9%)	11 (78.6%)	20 (74.1%)	1 (5.9%)
PEN	GEN	RIF†		1	1 (3.4%)	0	1 (3.7%)	0
PEN	SXT			1	1 (3.4%)	0	0	1 (5.9%)
PEN	FUS			2	1 (3.4%)	1 (7.1%)	0	2 (11.8%)
PEN	ERY	FUS	SXT†	2	2 (6.9%)	0	0	2 (11.8%)
PEN	GEN			4	3 (10.3%)	1 (7.1%)	2 (7.4%)	2 (11.8%)
PEN	TCY			2	2 (6.9%)	0	0	2 (11.8%)
BORSA								
OXA	PEN			1	1 (3.4%)	0	1 (3.7%)	0
OXA	PEN	FUS†		1	1 (3.4%)	0	0	1 (5.9%)
MRSA								
OXA	PEN			3	3 (10.3%)	0	2 (7.4%)	1 (5.9%)
OXA	PEN	FUS†		3	2 (6.9%)	1 (7.1%)	0	3 (17.6%)

BORSA = borderline oxacillin-resistant *Staphylococcus aureus*; ERY = erythromycin; FUS = fusidic acid; GEN = gentamicin; HIV = human immunodeficiency virus; MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S. aureus*; OXA = oxacillin; PEN = penicillin; RIF = rifampicin; SXT = sulfamethoxazole; TCY = tetracycline.

* This represents a subset of all the *S. aureus* isolates identified in the study.

† Denotes resistance to ≥ 3 antibiotics (selected antibiotic resistance results are shown).

likely to be TMP–SXT susceptible versus other SCCmec types.^{39,40} Further research is warranted to determine whether TMP–SXT may have a protective effect on *S. aureus* nasal colonization when used as *Pneumocystis jirovecii* prophylaxis among HIV-infected children.

Other factors impacting *S. aureus* nasal carriage. In addition to confirming that sociodemographic factors, including proximity to health-care facilities, predicted carriage, we found an association between prior pneumonia and *S. aureus* nasal carriage. Unfortunately, given the cross-sectional nature of the analysis, we were unable to determine the etiology of prior pneumonias and cannot infer a causal relationship between nasal colonization and pneumonia. Notably, TB was also associated with *S. aureus* carriage. Whether this association reflects a causal interaction between *S. aureus* colonization and TB disease or treatment of TB disease is also unclear. Prospective research is warranted to determine whether higher carriage rates in individuals with a history of TB reflects increased nosocomial transmission of *S. aureus* by children attending TB clinics. We found no evidence suggesting that colonization in TB patients had been driven by selection of rifampicin resistance, a key constituent of standard TB therapy and a drug with antistaphylococcal activity.

To our knowledge, this is one of the only studies in Africa to report the prevalence of intermittent and persistent *S. aureus* nasal carriage and investigate predictors of these carriage phenotypes. Given that we found that risk factors differ, and infection risk also differs by carriage status, this is an important strength of this study. Had our estimate of *S. aureus* nasal carriage relied on a single swab alone, we would have failed to detect carriage in 17% (*N* = 9) of children, since intermittent carriers, by definition, carry *S. aureus* only some of the time. Thus, 29% of carriers that were colonized only at the second visit, would have been misclassified as noncarriers, and we would have underreported prevalence as 39%. Since HIV-infected children were more likely to be persistently colonized, this bias would have primarily underreported carriage among HIV-uninfected children. Additionally, we used broth-enriched cultures to supplement agar plating, which can enhance the ability to detect *S. aureus*. Evidence suggests that enriched nasal cultures

increased sensitivity to *S. aureus* and MRSA screening more than direct plating alone, allowing for a more accurate determination of prevalence.¹⁸

Despite these strengths, we acknowledge that our conclusions are limited by the small sample size, particularly of HIV-infected children, an important and potentially high-risk group, older children, and HIV-exposed children. Nevertheless, given the striking carriage rate in HIV-infected children and prior evidence that HIV-infected individuals are at higher risk than others of MRSA autoinfection,^{13,41} further studies should evaluate *S. aureus* and MRSA carriage in that population to assess the risk of endogenous infection and explore potential reservoirs and sources of infection in the community. Finally, due to the cross-sectional design of our study and the fact that we relied on interview responses for much of our data, we cannot link carriage to subsequent infection and acknowledge that recall bias may have affected our results.

SUMMARY

In conclusion, we determined the prevalence of nasal colonization with *S. aureus* in an ambulatory pediatric population in southern Botswana to be 55%; nearly a third of these isolates were MRSA. *Staphylococcus aureus* colonization was associated with a history of pneumonia and a history of TB disease and, among HIV-infected children, HIV viremia. While MRSA prevalence was high, HIV status was not associated with increased risk of carriage. Future studies should address the role of nasal colonization and other body sites on subsequent infection and the utility of decolonization in that setting.

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