ORIGINAL ARTICLE

Nasopharyngeal colonization of Gambian infants by *Staphylococcus aureus* and *Streptococcus pneumoniae* before the introduction of pneumococcal conjugate vaccines

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Abstract

Staphylococcus aureus and *Streptococcus pneumoniae* commonly colonize the upper respiratory tract and can cause invasive disease. Several studies suggest an inverse relationship between these two bacteria in the nasopharynx. This association is of particular concern as the introduction of pneumococcal conjugate vaccines (PCVs) that affect pneumococcal nasopharyngeal carriage become widespread. A cohort of children in rural Gambia were recruited at birth and followed for 1 year, before the introduction of PCV into the routine immunization program. Nasopharyngeal swabs were taken immediately after birth, every 2 weeks for the first 6 months and then every other month. The presence of *S. aureus* and *S. pneumoniae* was determined using conventional microbiologic methods. Prevalence of *S. aureus* carriage was 71.6% at birth, decreasing with age to reach a plateau at approximately 20% between 10 to 20 weeks of age. Carriage with any *S. pneumoniae* increased during the first 10 weeks of life to peak at approximately 90%, mostly of PCV13 serotypes. Although in the crude analysis *S. aureus* carriage was inversely associated with carriage of any *S. pneumoniae* and PCV13 serotypes, after adjusting by age and season, there was a positive association with any carriage (odds ratio 1.32; 95% confidence interval 1.07–1.64; p 0.009) and no association with carriage of PCV13 serotypes (odds ratio 0.99; 95% confidence interval 0.70–1.41; p 0.973). Among Gambian infants, *S. aureus* and *S. pneumoniae* are not inversely associated in nasopharyngeal carriage after adjustment for age. Further carriage studies following the introduction of PCV are needed to better understand the relationship between the two bacteria.

Keywords: Carriage, infants, nasopharyngeal, *Staphylococcus aureus*, *Streptococcus pneumoniae*
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Introduction

Staphylococcus aureus and *Streptococcus pneumoniae* are two common colonizers of the upper respiratory tract that may cause severe invasive disease [1,2]. *S. aureus* is the leading cause of skin and soft tissue infections affecting individuals in the community and hospitals and also a leading cause of neonatal sepsis and childhood pneumonia [3,4]. *S. pneumoniae* is a frequent cause of pneumonia, meningitis and septicaemia [5]. For both bacteria, asymptomatic colonization (or carriage) of the nasopharynx is considered a necessary step on the pathway to disease [6–8].

S. aureus is found most frequently in the anterior nares but is also commonly found in the oropharynx and nasopharynx, on the skin and to a lesser extent in the gastrointestinal tract, perineum and axilla [9]. *S. pneumoniae* is carried primarily in the nasopharynx. Carriage of *S. pneumoniae* is most prevalent in children and older adults, while *S. aureus* shows a different age colonization pattern being highest among neonates

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[10,11] and then relatively constant in older age groups. Worldwide, approximately 20% to 30% of adults are nasal carriers of *S. aureus* [9] but there is wide variation in reports of *S. aureus* carriage rates among paediatric and surgical patient populations in sub-Saharan Africa, ranging from 14% in Ghana to 47% in South Africa [12]. The prevalence of *S. aureus* was highest among white men in one study which looked at healthy volunteer donors of different ethnic origins [13].

The introduction of pneumococcal conjugate vaccines (PCVs) alters the microbial flora in the nasopharynx of vaccinated individuals and their contacts. Although the overall prevalence of pneumococcal carriage has remained constant in most settings, there has been a substantial decrease of pneumococcal serotypes included in the vaccine, vaccine serotypes (VT) [14] and an increase in other serotypes not included in the vaccine, nonvaccine serotypes (NVT) [15,16]. One study found that *S. aureus* also increased after the introduction of PCVs [17], and several studies have reported an inverse association between asymptomatic carriage of *S. pneumoniae* and *S. aureus* [17–19]. In some instances this inverse association has been described in relation to specific pneumococcal serotypes [20,21].

There are increasing concerns about a potential increase in *S. aureus* carriage and disease after the introduction of PCVs into Africa [22]. In the Gambia, the epidemiology of pneumococcal carriage, before and after PCV introduction, has been well described [10,23]. However, little is known about the epidemiology of *S. aureus* nasopharyngeal carriage. Therefore, we used available data to study the epidemiology and risk factors for *S. aureus* nasopharyngeal carriage in a cohort of Gambian infants before the introduction of PCV and evaluated the association with *S. pneumoniae* carriage.

Methods

Study population

Infants were recruited as part of a large longitudinal carriage survey that was conducted in a rural area of western Gambia between 17 December 2003 and 16 June 2005 [10]. We used samples and data from infants who were part of an ancillary study that assessed the association between routine vaccination and bacterial carriage [24]. All infants were vaccinated according to the Gambia expanded program of immunization's schedule which then offered bacillus Calmette-Guérin, oral polio, pentavalent (diphtheria, pertussis, tetanus hepatitis B and *Haemophilus influenzae* type b), measles and yellow fever vaccines. PCV was introduced into the Gambian expanded program of immunization in 2009.

Sample collection

Nasopharyngeal swab samples were taken from infants as soon as possible after birth, every 2 weeks for the first 6 months and then every other month until their first birthday. Swab samples were collected, transported and stored according to World Health Organization guidelines [25].

Swabs were collected from the posterior wall of the nasopharynx using a calcium alginate swab and immediately placed in vials containing skim milk–tryptone–glucose–glycerol (STGG) transport medium. Vials were then transported within 8 hours of collection to the Medical Research Council (MRC) Unit The Gambia, Fajara, laboratories and stored at −70°C.

Isolation of bacteria

Samples were tested in batches for *S. pneumoniae* as part of the initial study [10]. To isolate *S. pneumoniae*, 10 μL of a STGG sample were streaked onto a gentamicin blood agar plate and incubated for 18 to 24 hours at 35°C in 5% CO_2. Pneumococci were identified on the basis of colony morphology, optochin susceptibility and/or bile solubility. Serotyping was done with capsular and factor typing sera (Statens Serum Institute, Copenhagen) using the latex agglutination technique [26]. Isolates with equivocal serotype results were confirmed by the Quellung reaction.

In 2012, the STGG samples were plated again onto agar plates for the isolation of *S. aureus* for the ancillary study. To isolate *S. aureus*, 50 μL of thawed STGG sample were plated onto mannitol salt agar plates and incubated for 48 hours at 37°C in ambient temperature. The plates were examined for yellow or white colonies typical of staphylococci and subcultured onto blood agar plates to obtain pure growth. All suspected colonies were tested with a catalase test, followed by coagulase testing when this was positive, using the Remel Staphaurex Plus kit (OXR30950201; Oxoid) to confirm the identity of *S. aureus*.

Statistical analysis

We analysed trends in the prevalence of *S. aureus* and *S. pneumoniae* carriage by splitting the sample into 10 groups of equal size, with the first group containing 10% of the samples taken at the youngest ages, the second group containing those taken at the next youngest 10% and so on. In addition, we fitted spline functions to the prevalence data as previously described [24].

We used logistic regression to model the association between *S. aureus and S. pneumoniae* carriage (all serotypes, 13-valent PCV (PCV13) VT and PCV13-NVT) adjusted for season and age. Season was included in the model as a binary factor (dry season from June to October and rainy season from November to May), and age was included as a restricted cubic spline. Other risk factors including sex, breast-feeding, upper

respiratory tract infection (URTI) and ear discharge at the time of swabbing, antibiotic use and travel out of the study area in the previous 2 weeks were also examined. Confidence intervals (CIs) were adjusted for clustering at the village level using robust standard errors.

We conducted a secondary analysis using conditional logistic regression to estimate the association between *S. aureus and S. pneumoniae* carriage within individuals, thereby eliminating any confounding attributable to variation between individuals.

Ethical approval

The cohort study was approved by the joint MRC–Gambia Government (GG) ethics committee and by the ethics committee of the London School of Hygiene and Tropical Medicine; the subsequent study that collected additional vaccination data was also approved by the MRC GG ethics committee. Written consent was obtained from the parents or guardians of each infant recruited into the study.

Results

Characteristics of study population

A total of 237 infants were recruited into the original study. Here, we included all infants from the previous ancillary study that had data on carriage of *S. aureus* and *S. pneumoniae* (i.e. 57.6% of the original cohort); an additional 11 infants were excluded from that study because their vaccination record was incomplete [24]. Altogether, 147 infants were included in our analysis, providing a total of 1873 samples with median of 14 samples per infant (range, 2–17). Overall, 54.4% of these infants were boys. The median ages at which routine vaccines were given was 1.9 weeks (interquartile range (IQR) 1.4–2.4) for bacillus Calmette-Guérin, 26.3 weeks (IQR 22.7–33.3) for the third dose of the pentavalent vaccine, 43.1 weeks (IQR 40.6–48.1) for measles vaccine and 44.0 weeks (IQR 40.9–48.9) for yellow fever vaccine. The majority of infants were breast-fed; only on 7/1753 occasions was the infant not breast-feeding at the time of swabbing.

Prevalence of *S. aureus* and *S. pneumoniae*

Of the 1873 samples, 30.9% were positive for *S. aureus*, 82.0% positive for *S. pneumoniae* and 23.2% positive for both bacteria. The 1535 *S. pneumoniae*–positive samples yielded 1673 isolates with a total of 65 serotypes/groups. Among all samples collected, 51.2% were positive for PCV13-VT and 34.1% were positive for PCV13-NVT (including 2.9%, *n* = 49, nontypeable pneumococci).

Prevalence of *S. aureus* was highest immediately after birth at 74.5% in the seven samples taken within the first 24 hours of life and decreased to 21.4% by 20 weeks of age. Prevalence of *S. pneumoniae* carriage, on the other hand, was low at birth, increased rapidly with age and reached a plateau after 10 weeks of age (approximately 90%). In infants older than 20 weeks, the prevalence of carriage of both bacteria remained constant until the end of the follow-up at 1 year of age (Fig. 1). The age pattern of PCV13-VT carriage was similar to that for overall pneumococcal carriage, but the peak was observed in older infants at approximately 40 weeks of age. For PCV13-NVT, the peak of 51% carriage occurred very early (8–9 weeks of age), and carriage subsequently decreased to about 25% after 34 weeks (Fig. 1).

Risk factors for *S. aureus*

A crude analysis showed an inverse association between carriage of *S. aureus* and *S. pneumoniae* (inverse association with any pneumococcal carriage and PCV13-VT carriage) and also with age (Table 1). After adjusting for age and season, *S. aureus* was positively associated with carriage of any *S. pneumoniae* (odds ratio (OR) 1.32; 95% CI 1.07–1.64; p 0.009) but was no longer associated with carriage of PCV13-VT (OR 0.99; 95% CI 0.70–1.41; p 0.973). In an analysis using conditional regression, the statistical significance of the increased risk of any *S. pneumoniae* colonization among *S. aureus* carriers after adjusting for age and season was borderline (adjusted OR 1.41; 95% CI 0.99–2.03; p 0.059).

Apart from URTI, which was negatively associated with carriage, none of the other risk factors tested in the adjusted analysis was associated with carriage of *S. aureus* (Table 1).

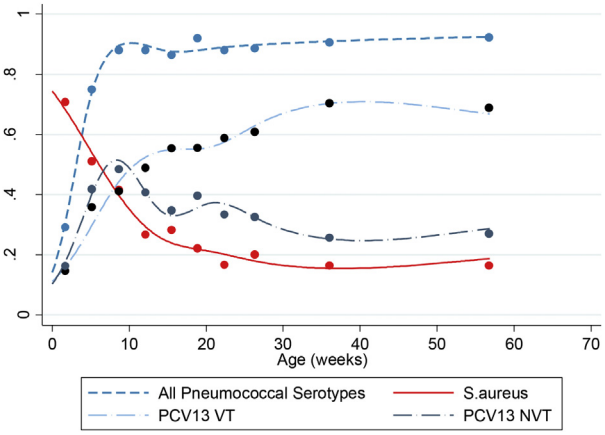

FIG. 1. Prevalence of *Staphylococcus aureus* and *Streptococcus pneumoniae* (all serotypes, VT and NVT) by age. Dots indicate prevalence in each decile of age; lines, predictions from logistic regression where effect of age on carriage is modelled using spline functions. VT, vaccine serotype; NVT, nonvaccine serotype.

TABLE 1. Risk factors for colonization with *Staphylococcus aureus* and *Streptococcus pneumoniae*

				Unadjusted		Adjusted	
Risk factor	**Variable**	***n***	***S. aureus, n* (%)**	**OR (95% CI)**	**p**	**OR[a]**	**p**
S. pneumoniae							
Any serotype	No	338	144 (42.6)	1		1	
	Yes	1535	435 (28.3)	0.53 (0.41–0.70)	<0.001	1.32 (1.07–1.64)	0.009
PCV13-VT	No	915	334 (36.5)	1		1	
	Yes	958	245 (25.6)	0.60 (0.48–0.75)	<0.001	0.99 (0.70–1.41)	0.97
PCV13-NVT	No	1235	376 (30.4)	1		1	
	Yes	638	203 (31.8)	1.07 (0.82–1.39)	0.64	1.13 (0.78–1.63)	0.53
Sex	F	865	267 (30.9)	1		1	
	M	1008	312 (32.0)	1.00 (0.79–1.28)	0.97	0.98 (0.76–1.27)	0.90
Season	Rainy	738	176 (23.8)	1		1	
	Dry	1135	403 (35.5)	1.76 (1.30–2.38)	<0.001	1.26 (0.87–1.81)	0.22
Breast-fed	Mixed	643	117 (18.2)	1		1	
	Exclusive	1103	381 (34.5)	2.37 (1.92–2.92)	<0.001	1.05 (0.77–1.42)	0.77
URTI	No	1673	482 (28.8)	1		1	
	Yes	79	15 (19.0)	0.58 (0.38–0.88)	0.01	0.58 (0.41–0.84)	0.003
Ear discharge	No	1745	495 (28.4)	1		1	
	Yes	8	2 (25.0)	0.84 (0.20–3.51)	0.82	1.60 (0.36–7.08)	0.54
Antibiotic[b]	No	1588	460 (29.0)	1		1	
	Yes	112	24 (21.4)	0.67 (0.40–1.11)	0.12	0.70 (0.42–1.18)	0.19
Travel out[b]	No	1236	358 (29.0)	1		1	
	Yes	341	75 (22.0)	0.69 (0.53–0.91)	0.008	0.90 (0.70–1.17)	0.43
Age	<12 weeks	644	332 (51.6)	1			
	12–24 weeks	660	148 (22.4)	0.27 (0.22–0.34)	<0.001		
	>24 weeks	569	99 (17.4)	0.20 (0.15–0.26)	<0.001		

CI, confidence interval; NVT, nonvaccine serotype; OR, odds ratio; PCV, pneumococcal conjugate vaccine; URTI, upper respiratory tract infection; VT, vaccine serotype.
[a]Adjusted for season and age as continuous percentiles.
[b]Previous 2 weeks.

Discussion

In this study, we demonstrated that the inverse association between *S. aureus* and *S. pneumoniae* nasopharyngeal carriage among Gambian infants before the introduction of PCV is explained by the difference in age pattern of carriage during the first year of life, and we found that the confounding effect of age masked a potential positive association between *S. aureus* and *S. pneumoniae*.

Several studies have shown an inverse association between *S. aureus* and *S. pneumoniae* in Africa and other regions, including studies by Bogaert *et al.* [20] in Dutch children aged 1 to 19 years, and Madhi *et al.* [27] in South Africa among HIV-uninfected children about 5 years of age. One of them did adjust for age. In a randomized controlled trial in the Netherlands which recruited children aged 1 to 7 years with recurrent otitis media, *S. aureus* carriage was higher in children who received pneumococcal vaccines compared to placebo [28]. Regev-Yochay *et al.* [21] found an inverse association that persisted after adjustment for age among children younger than 40 months old (median, 1.3 years) in Israel. There are differences between the latter study and ours in that 80% of the children in the Regev-Yochay *et al.* study had respiratory tract infections which could alter the normal flora in the nasopharynx [29]. We also swabbed the children more frequently and included age as a continuous variable in our analysis. In another Gambian study, an inverse association between overall pneumococcal carriage and *S. aureus* carriage also disappeared in the adjusted analysis [19].

A positive association between *S. aureus* and *S. pneumoniae* has not previously been reported, although Shiri *et al.* [18] noted increased *S. pneumoniae* colonization among individuals with dual carriage of *H. influenzae* and *S. aureus*. A possible explanation for this association is that susceptibility to carriage varies within individuals over time, and this variation affects the risk of acquiring both *S. aureus* and *S. pneumoniae*. The positive association is not attributable to variation between individuals in susceptibility to carriage because the association was also observed in the within-individual analysis.

Our study showed a very high prevalence of *S. aureus* nasopharyngeal carriage among Gambian infants, as previously reported in another setting in the country [30]. Approximately 70% of newborns carried *S. aureus*; the prevalence decreased to 20% between 10 and 20 weeks of life. A rapid drop in prevalence during this first year of life has been reported previously [31], although possibly later in infancy, as shown among PCV-unvaccinated infants [17]. We found that the age–carriage trend for VT pneumococci, but not NVT pneumococci, was similar to the trend for any pneumococcal carriage. This may explain why van Gils *et al.* [17] found an inverse association between *S. aureus* and VT pneumococci was stronger than that between *S. aureus* and NVT pneumococci.

Besides being positively associated with *S. pneumoniae*, we found that carriage of *S. aureus* was negatively associated with URTI. The reason for this association is unclear, but it might be

due to competitive interaction with other bacteria and possibly viruses which we did not explore. Carriage of *S. aureus* was not associated with breast-feeding, season or antibiotic use. The absence of an association with breast-feeding is consistent with the findings of a large cohort study [31]. On the other hand, the absence of an association with antibiotic use is not consistent with previous studies which have shown that recent antibiotic use lowers *S. aureus* carriage [32], but it is unsurprising because antibiotics were rarely used among infants in our study. Seasonality of *S. aureus* carriage has not been documented, although the prevalence of *S. pneumoniae* carriage in children increases during the dry season [33]. Other factors including crowding and socioeconomic status may also be important predictors of bacterial carriage [34], but these data were not collected in the original study.

S. aureus is primarily carried in the anterior nares; therefore, carriage of *S. aureus* might have been more frequently detected if nasal or oral swabs had been used in addition to nasopharyngeal swabs. In a recent study of Gambian infants, *S. aureus* was isolated from 65% of oropharyngeal swabs, but only 36% of nasopharyngeal swabs [30]. We may therefore have underestimated the prevalence of *S. aureus*. However, we would not expect this to alter the direction of the association between *S. aureus* and *S. pneumoniae*. The culture for *S. aureus* was done several years after initial sample storage. Hare *et al.* [35] have shown that other respiratory pathogens remain viable when cultured from original swabs stored in STGG at −70°C for up to 12 years. *S. aureus*, though not one of the bacteria in their study, is a very robust organism and there is no reason to believe it would behave differently.

In assessing the relationship between *S. aureus* and *S. pneumoniae*, age should be considered as an important confounder, at least among infants. After controlling for the effect of age, our study found a positive association between these two pathogens in the nasopharynx of infants, which suggests that *S. aureus* will not increase in prevalence after the introduction of PCV. However, further surveys of bacterial carriage after introduction of PCVs in the Gambia are required to confirm this prediction.

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Conflict of Interest

None declared.

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