Original Article

High prevalence of methicillin-resistant Staphylococcus aureus carriage among infants at the Children’s Hospital, Accra, Ghana

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Abstract

Introduction: Infants are at risk of Staphylococcus aureus (S. aureus) colonization and infection. The aim of this study was to investigate S. aureus and methicillin-resistant S. aureus (MRSA) colonization among infants, including the prevalence, predictors of colonization, and antibiogram.

Methodology: The study was cross-sectional, and involved infants aged less than one year recruited at the Princess Marie Louise Children’s Hospital in Accra, Ghana. Sociodemographic and clinical data of the participants were gathered with a structured questionnaire. Nasal swabs were also obtained from them and bacteriologically cultured. S. aureus was confirmed with the coagulase test, and MRSA was confirmed by polymerase chain reaction (PCR) of the mecA gene. Antimicrobial susceptibility testing of S. aureus was done using the Kirby-Bauer method.

Results: The carriage prevalence of S. aureus and MRSA were 34.9% (45/129) and 17.10% (22/129), respectively. Colonization with coagulase-negative Staphylococci (CoNS) was protective of both S. aureus (OR = 0.008; p < 0.001) and MRSA (OR = 0.052; p = 0.005) carriage. Maintenance of good hand hygiene prevented S. aureus carriage (OR = 0.16; p < 0.001).

S. aureus resistance to antibiotics decreased across penicillin (96%), trimethoprim-sulfamethoxazole (61%), tetracycline (61%), erythromycin (39%), gentamicin (39%), fusidic acid (26%), rifampicin (17%), clindamycin (7%), and linezolid (0%); 68.8% S. aureus were multidrug resistant.

Conclusions: S. aureus and MRSA prevalence were high among the infants. Colonization with CoNS and good hand hygiene maintenance were predictive of MRSA and methicillin-sensitive S. aureus (MSSA) colonization, respectively.

Key words: Multidrug resistant; Staphylococcus aureus; MRSA; Infants; PCR; mecA.

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Introduction

Staphylococcus aureus (S. aureus) preferentially colonizes the moist squamous epithelium of the anterior nares, and less frequently, other anatomical sites [1]. The clinical significance of its colonization is reflected in it being an antecedent to subsequent infections such as endocarditis, pneumonia, septicemia, and meningitis [2, 3]. Moreover, the treatment of its infections has been complicated by the occurrence and spread of its multidrug resistant strains, particularly, those referred to as methicillin-resistant S. aureus (MRSA), which are refractory to all antibiotics of the beta-lactam class – a trait that their methicillin-sensitive contemporaries called methicillin-sensitive S. aureus (MSSA) lack [4]. The molecular basis of this methicillin resistance is the encoding of a novel penicillin-binding protein with lower affinity to penicillin – PBP2a – by the acquired cassette-borne mecA gene and its variants thereof, such as the recently discovered mecC gene [4]. Similar to some other pathogens such as Clostridium difficile, biofilm formation occurs extensively in some S. aureus strains, and this contributes to the relatively high levels of antibiotic resistance in MRSA [5,6,7]. Besides the mec genes that mediate multidrug resistance in S. aureus, several virulence genes of the pathogen have been
identified, including the Panton-Valentine leukocidin (pvl) gene, whose gene product causes lysis of polymorphonuclear white blood cells and tissue necrosis [7,8]. Although the pvl gene typically occurs in community-associated MRSA, it is frequently encountered among S. aureus strains circulating in Africa, including methicillin-sensitive ones [9–12]. Given the potential high clinical significance of the pathogen among them, this study investigated S. aureus and MRSA colonization among infants, focusing on prevalence and risk factors for carriage, as well as the pvl gene carriage of MRSA and the antibiogram of colonizing S. aureus.

Methodology

Study site, design, and sampling

This study was carried out using the Princess Marie Louise Children’s Hospital (PML) in Accra as the study site. Accra is the capital city of Ghana, and is home to approximately two million individuals, as well as eight government or quasi-governmental hospitals (http://www.statsghana.gov.gh/). Besides the Korle Bu Teaching Hospital, which includes numerous specialties and provides specialized paediatric care, PML is the only other major public hospital in Accra that has pediatric care as its focus. This was a cross-sectional study involving 129 healthy infants visiting the outpatient clinic of the hospital, either for postnatal care or by virtue of accompanying their guardians for their routine medical examinations. Sampling of the participants was done between January and July 2017, and their parents/guardians gave consent for their participation. The exclusion criteria were: being on antimicrobials two weeks prior to sampling and presence of comorbidities (with the exception of asthma, which was found in two of the study participants). Data on potential S. aureus and MRSA carriage determinants were gathered from the infants using a standard questionnaire.

A qualified paediatrician rotated a sterile swab stick in both anterior nares of each of the infants. The resultant swab specimens were maintained on ice in uniquely-labeled 1 mL skim milk-tryptone-glucose-glycerin (STGG) medium-containing vials, and after four hours following collection, they were transported to the Department of Medical Microbiology, University of Ghana Medical School, for laboratory processing. Each of the swab specimens were vortexed for approximately two minutes, and thereafter refrigerated at -80 °C, until needed.

Laboratory Analysis

The procedures described by Donkor et al. [24] and a few modifications thereof guided the specimen processing, S. aureus and MRSA identification, antimicrobial susceptibility testing, and molecular
Bacteriological culture was done on mannitol salt agar. Staphylococcal isolates were presumptively identified based on their reaction to Gram stain, catalase, and the tube coagulase test. Coagulase-negative isolates were identified as *S. aureus*, and screened for resistance to tetracycline (30 µg), erythromycin (15 µg), gentamicin (10 µg), rifampicin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), penicillin (10 µg), clindamycin (2 µg), fusidic acid (10 µg), linezolid (10 µg), and cefoxitin (30 µg). The antimicrobial resistance screening was conducted, and the results interpreted, in line with the Clinical and Laboratory Standards Institute guidelines [31]. Subsequently, the cefoxitin-resistant isolates were subjected to polymerase chain reaction (PCR) targeting the *mecA*, *nucA*, and *pvl* genes. Cefoxitin-resistant, *mecA*-positive, isolates were identified as MRSA.

The molecular methods used in the investigation of the isolates are as follows: The manufacturer’s instructions of the Zymo Research extraction kit (Zymo Research Corp., Irvine, USA) were used as a guide to extract genomic DNA from overnight lysogenic broth cultures of a *S. aureus* positive control strain (ATCC 25923) and the MRSA isolates. In order to check the quality of DNA, 5 µL of each extracted DNA was mixed with 2 µL of bromophenol blue gel loading buffer and ran on a 1.2% agarose gel; the resultant bands were visualized by UV illumination. The extracted genomic DNA was used as template for the PCR amplification of each of the three genes – *mecA*, *nucA*, and *pvl* – in a total reaction volume of 50 mL, made up of the genomic DNA (60 ng/µL final concentration), deoxyribonucleoside triphosphates (dNTPs) (200 µM final concentration), MgCl2 (2 mM final concentration), *Taq* polymerase (1.25 U/µL final concentration), primers (0.2 µM final concentration), and PCR water; RNase-free water was used as the negative control. The primer sequences used in the amplification of the *mecA*, *nucA*, and *pvl* genes were those described by Sajith Khan et al. [32], Brakstad et al. [33], and Deurenberg et al. [34], respectively. Separation of the amplicons was done using 1.2% agarose gel electrophoresis, followed by visualization with the aid of UV illumination.

### Data Analysis

The data were analyzed using STATA 14 (Strata Corp, College Station, TX, USA). Descriptive statistics were used to summarize data on the resistance of *S. aureus* to the tested antimicrobials. A combination of independent samples Chi square tests, point biserial correlations, and binary logistic regression were used to identify determinants of *S. aureus* and MRSA colonization. The significance of each determinant was evaluated using its *p* value, odds ratio, and confidence interval; *p* values whose magnitudes were below 0.05 were deemed significant.

### Ethical Approval

This study was approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana, with protocol identification number “CHS-Et/M.3 – P 4.4/2016-2017”.

### Results

#### Demographic, household, and clinical features of the infants

The one hundred and twenty-nine (129) infants recruited as participants in this study were predominantly male (58.1%, *n* = 75), and had a mean age of 18.29 weeks. Furthermore, majority of them were assisted by their caregivers to maintain good hand hygiene (55.8%, *n* = 72), resided in compound houses (86%, *n* = 111), with an average of 4.19 persons living in these households, but did not have any member of their households employed in healthcare (100%, *n* = 129).
A summary of the demographic and household characteristics of the infants is presented in Table 1.

The majority of the participants had not been hospitalized (67.4%, n = 87), and had no history of surgery (98.4%, n = 127), asthma (98.4%, n = 127), or pneumonia (98.4%, n = 127). The clinical features of the infants are presented in Table 2.

**Carriage of S. aureus and MRSA, and associated determinants**

In the current study, 17.1% (n = 22) of the infants carried MSSA exclusively, and 17.8% (n = 23) carried MRSA exclusively, making the total carriage prevalence of *S. aureus* 34.9% (n = 45); none of the participants had MRSA-MSSA co-colonization. The carriage prevalence of *pvl*-positive MRSA was 16.28 (n = 21); hence the proportion of MRSA isolates that were *pvl*-positive was 84% (21/25). Conversely, coagulase-negative Staphylococci (CoNS) were present in 40.3% (n = 52) of the infants, and none of them concurrently carried *S. aureus*.

The logistic regression analysis revealed that of the variables evaluated as risk factors for colonization, colonization with CoNS was protective of both *S. aureus* and MRSA colonizations. Moreover, maintenance of good hand hygiene was also found to be protective of *S. aureus* colonization, specifically, *S. aureus* isolates that are methicillin-sensitive, but not methicillin-resistant. The results of the risk factor analysis are presented in Table 3.

**Antibiogram of the S. aureus isolates**

The highest rate of antimicrobial resistance was recorded against penicillin, whereas no resistance was recorded against linezolid. Moreover, the proportion of *S. aureus* isolates that were multidrug resistant (those that were resistant to three or more classes of antimicrobials) [24] was 68.8%. The prevalence of antimicrobial resistance among the *S. aureus* isolates are presented in Figure 1.

**Discussion**

The current study investigated the epidemiology of *S. aureus* and MRSA nasal carriage among infants. It is the first of its kind in the country, and among the few such studies in this risk group globally. The carriage prevalence of *S. aureus* was found to be 34.9%, and the respective prevalence for MSSA and MRSA were 17.1% and 17.8% respectively. The *S. aureus* and MRSA carriage prevalence seem higher than those reported in a study conducted among children in the country – 22.1% and 2%, respectively [30]. This difference might be accounted for by the fact that Eibach et al.’s study [30] included children aged up to 129).

![Antimicrobial resistance in the S. aureus isolates.](Figure 1)

| Risk factor                   | *S. aureus colonization OR (95% CI) | p value | MSSA colonization OR (95% CI) | p value | MRSA colonization OR (95% CI) | p value |
|------------------------------|----------------------------------|---------|-----------------------------|---------|-------------------------------|---------|
| Colonization with CoNS       | 0.008 (0.001–0.069)              | < 0.0001| N/A                         | N/A     | 0.052 (0.007–0.403)            | 0.005   |
| Practice of good hand hygiene| 0.055–0.476                      | 0.001   | 0.176 (0.059–0.524)         | 0.002   | N/A                           | N/A     |

CoNS: Coagulase-negative Staphylococci; N/A: Not applicable.

Table 3. Determinants of *S. aureus* and MRSA colonization.

Table 2. Clinical features of the infants.

| Clinical feature                      | N (%) |
|--------------------------------------|-------|
| Self-medication of parents           |       |
| Yes                                  | 42 (32.6) |
| No                                   | 87 (67.4) |
| Hospital admission                   |       |
| Yes                                  | 42 (32.6) |
| No                                   | 87 (67.4) |
| History of surgery                   |       |
| Yes                                  | 2 (1.6) |
| No                                   | 127 (98.4) |
| History of asthma                    |       |
| Yes                                  | 2 (1.6) |
| No                                   | 127 (98.4) |
| History of pneumonia                 |       |
| Yes                                  | 2 (1.6) |
| No                                   | 127 (98.4) |

Duration of hospital admission: 1.14 ± 2.21 days.
more than ten years, a wider age range than that in this study which focused on infants (<1 yrs). Indeed, age has been identified as an important determinant of *S. aureus* carriage [30,35]. Another noteworthy observation that makes the disparity in prevalence between the current study and that of Eibach et al. [30] quite paradoxical is that the participants sampled in that study were admitted in the hospital with a variety of microbial infections and hospital admission is a known risk factor for *S. aureus* carriage [23,27,36,37]. The *S. aureus* prevalence recorded in the current study is lower than the 49% recorded by Donkor & Nartey [29] in their study involving children under five years of age, and this could reflect a temporal decline in *S. aureus* carriage, given that sampling of the participants for the two studies were done about a decade apart. This may especially be the case, as nasal carriage prevalence of 10–23.4% has been reported among healthy individuals in more recent studies [24,27,36].

A high proportion (84.1%) of the MRSA isolated in the current study harboured the *pvl* gene. Although the MSSA isolates were not screened to determine their carriage of the gene due to limited resources, it is recognized that this high proportion of *pvl*-positive MRSA feeds into the widely accepted hallmark of *S. aureus* strains circulating in Africa – that they frequently harbour *pvl* [9–12]. Moreover, carriage of the *pvl* gene is often associated with CA-MRSA [38,39].

In the current study, colonization with CoNS was found to be protective of both *S. aureus* and MRSA colonization, indicating that absence of colonization with these organisms predisposes to carriage of *S. aureus* and MRSA. Donkor et al. [24] made a similar finding, albeit among HIV-infected children. The finding is further supported by the reports of Iwase et al. [40], Olson et al. [41], and Paharik et al. [42]. Iwase et al. [40] and Olson et al. [41] demonstrated that *Staphylococcus* epidermis, a part of CoNS, prevents *S. aureus* colonization. Paharik et al. [42] provided the mechanism underlying this antagonism – CoNS prevents *S. aureus* colonization by inhibiting agr quorum sensing in the latter using its autoinducing peptide. This suggests that the autoinducing peptide, as well as other CoNS-produced peptides that are cidal to *S. aureus*, could be further investigated for their usefulness in *S. aureus* decolonization strategies.

Maintenance of good hand hygiene was also found to be protective of *S. aureus* colonization, specifically, *S. aureus* isolates that are methicillin-sensitive, but not methicillin-resistant – this distinction regarding the protective value of good hand hygiene against bacterial colonization is an intriguing finding. Overall, it is at odds with the study conducted by Donkor et al. [24] cited above, which reported that regular hand washing with soap and a good hand hygiene practice increased MRSA colonization by more than six folds, with a possible link to the use of triclosan-containing soap. It is noted that in the current study the investigation of good hand hygiene practice did not make a distinction between the use of soaps for hand washing and the application of hand sanitizers and other preparations made for the promotion of hand hygiene. That notwithstanding, this finding fits into the long-held custom of promoting good hygiene practices as a means of controlling the spread of infections of several pathogens [43–48]. It is therefore imperative to intensify the promotion of good hand hygiene practice, particularly, among populations who are at a higher risk of *S. aureus* carriage.

The high rates of resistance that were recorded against penicillin, trimethoprim-sulfamethoxazole, tetracycline, fusidic acid, erythromycin, and gentamycin are consistent with those reported in other studies in the country [24,27,36], and can be attributed to their increased use over the years. It is interesting that our study revealed a high prevalence of trimethoprim-sulfamethoxazole resistance among *S. aureus* (which are probably community-associated), contrary to the observation that trimethoprim-sulfamethoxazole resistance tends to be more often linked with hospital-associated *S. aureus* [49,50]. This observation could be due to a number of reasons; the widespread routine use of trimethoprim-sulfamethoxazole in hospital and community settings has generally promoted resistance to the antibiotic among hospital and community-associated *S. aureus* isolates circulating in the country. The observation may also reflect a changing epidemiology of *S. aureus* with respect to hospital and community settings. Further studies using molecular methods are required to further understand this. The low resistance rate (0%) recorded against linezolid was expected, given that the drug has a low coverage, and access to it is restricted. The low rate also suggests that the drug could retain its place as part of the mainstays of anti-MRSA therapy. However, given the high proportion (68.8%) of multidrug resistant *S. aureus* in the current study, it would not be surprising if subsequent studies in the country begin reporting increasing resistance rates against the antimicrobial. It is therefore important to intensify antimicrobial stewardship programmes in the country.

This study was potentially limited by a few factors. First, molecular typing of the isolates was not done due...
Conclusions

It is concluded that the prevalence of *S. aureus* and MRSA were high among the infants, who may serve as reservoirs of multidrug resistant *S. aureus*. Colonization with coagulase-negative *Staphylococci* is a determinant of both *S. aureus* and MRSA colonization, while maintenance of good hand hygiene is a determinant of MSSA colonization. The autoinducing peptide produced by coagulase-negative *Staphylococci* could be further investigated for its usefulness in *S. aureus* decolonization strategies.

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