

Relationship between antibiotic resistance and sickle cell anemia: preliminary evidence from a pediatric carriage study in Ghana

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Background: Antibiotics are frequently used among people with sickle cell anemia (homozygous SS or HbSS disease), especially for prophylaxis. However, the relationship between antibiotic resistance and people with HbSS disease has not been adequately studied, especially in the developing world. The objectives of the study were (1) to compare antibiotic resistance patterns of nasal *Staphylococcus aureus* between children with HbSS disease and children without HbSS disease (healthy children) and (2) to evaluate nasopharyngeal carriage of antibiotic-resistant *Streptococcus pneumoniae* among children with HbSS disease.

Methods: This was a prospective cross-sectional study, and the subjects were children under 12 years old. Nasal swabs were collected from 50 children with HbSS disease and 50 children without HbSS disease. Nasopharyngeal swabs were collected from another group of 92 children with HbSS disease. The nasal and nasopharyngeal swabs were cultured for *S. aureus* and *S. pneumoniae*, respectively. Susceptibility testing was carried out on the *S. aureus* and *S. pneumoniae* isolates for various antibiotics, including penicillin, ampicillin, cefuroxime, erythromycin, cloxacillin, and cotrimoxazole.

Results: The carriage rates of *S. aureus* among pediatric subjects with HbSS disease and those without HbSS disease were 48% and 50%, respectively ($P > 0.05$). *S. pneumoniae* carriage among the pediatric subjects with HbSS disease was 10%. Antibiotic resistance patterns of *S. aureus* carried by children with HbSS disease and children without HbSS disease were similar, and the *S. aureus* resistance rates were $>40\%$ for the various antibiotics, with the exception of erythromycin and cloxacillin. Low levels of *S. pneumoniae* resistance (0%–11%) were observed for the various antibiotics tested except cotrimoxazole, which showed an extremely high-percentage resistance (100%).

Conclusion: Sickling status is not a risk factor for carriage of *S. aureus*. In this cohort of Ghanaian children with HbSS disease, *S. aureus* is higher in carriage and more antibiotic-resistant, compared to *S. pneumoniae*.

Keywords: Paediatric, sickle cell anaemia, antibiotic resistance, Ghana

Introduction

Antibiotic resistance has been attributed to the misuse of antibiotics, which exert selective pressure favoring the emergence of resistant strains.^{1,2} Antibiotic use selects for resistance not only in pathogenic bacteria, but also in the commensal flora of exposed individuals. Studies of the prevalence of antibiotic resistance in commensal microflora are vital in monitoring and understanding the process of antimicrobial-mediated selection in a population.^{3,4} Clinically, antibiotic resistance in commensal microflora is of significance because such organisms could cause opportunistic infections, as in immunosuppression or in underlying diseases such as sickle cell disease.⁵

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Antibiotics are frequently used among people with homozygous SS (HbSS) disease, especially for prophylaxis. However, the relationship between antibiotic resistance and people with HbSS disease has not been adequately studied, especially in sub-Saharan Africa. In Ghana, one study investigated *Streptococcus pneumoniae* penicillin resistance among people with HbSS disease.⁶ However, the study did not include a control arm, which makes it difficult to deduce any trend in antibiotic resistance that may be peculiar to people with HbSS disease. Recently, a Ugandan study showed that *S. pneumoniae* carried by people with HbSS disease were highly resistant to penicillin and cotrimoxazole, compared to a control group, but no significant variations were observed for chloramphenicol, erythromycin, ceftriaxone, rifampin, or perfloracin.⁷ Carriage studies that have investigated antibiotic resistance among people with HbSS disease in sub-Saharan Africa seem to have focused on *S. pneumoniae*,⁶⁻⁸ which is probably due to the prominence of the organism in bacteraemia in HbSS disease in Africa. However, some studies indicate that *Staphylococcus aureus* could be more important in invasive disease among people with HbSS disease than *S. pneumoniae*.⁹⁻¹³ So far, very few studies have investigated the antibiotic resistance of *S. aureus* carried by people with HbSS disease in sub-Saharan Africa, probably because there are few reports of *S. aureus*-related clinical diseases in children with HbSS in the region. Such a study would contribute to our understanding of the epidemiology of *S. aureus* diseases among people with HbSS disease in the region, and allow improvements in antimicrobial therapy for such infections.

In this study, we present preliminary carriage data on *S. aureus* and *S. pneumoniae* among children with HbSS disease in Ghana. The objectives of the study were (1) to compare antibiotic resistance patterns of nasal *S. aureus* between children with HbSS disease and children without HbSS disease (healthy children) and (2) to evaluate nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae* among children with HbSS disease.

Material and methods

Study area and sampling

The study was carried out at the pediatric unit of Korle-Bu Teaching Hospital in Accra, the capital city of Ghana, from February 2006 to April 2007. Korle-Bu Teaching Hospital is the largest hospital in Ghana, and the pediatric unit has a sickle cell clinic that runs once a week. The study was a cross-sectional study of randomly selected pediatric subjects under 12 years who were attending the hospital for reviews or

registration for the first time. Based on 95% confidence limits with an allowable error of 10%, 100 subjects, including 50 who had HbSS disease and 50 without HbSS disease (healthy children), took part in the *S. aureus* carriage study, while a second group of 92 children who had HbSS disease took part in the *S. pneumoniae* carriage study. The diagnosis of HbSS was based on hemoglobin electrophoresis. In the *S. aureus* carriage study, nasal specimens were collected from the study participants, while nasopharyngeal specimens were collected from the study participants in the *S. pneumoniae* carriage study. The specimens were collected with the aid of swabs and processed immediately in the laboratory.

Bacteria isolation and antibiotic susceptibility testing

The swab specimens were cultured for *S. aureus* and *S. pneumoniae* using standard microbiological methods.¹⁴ The specimens were inoculated onto plates of blood agar (Oxoid Ltd, Basingstoke, UK) and incubated at 37°C for 18–24 hours. Plates for *S. aureus* were incubated aerobically, while plates for *S. pneumoniae* were incubated in 5% CO₂.^{14,15} Suspected *S. aureus* isolates were confirmed using the coagulase test,¹⁴ while suspected *S. pneumoniae* isolates were confirmed using the optochin test.^{14,16} The confirmed isolates of *S. aureus* and *S. pneumoniae* were purified and subjected to antibiotic susceptibility testing using a modified form of the Kirby Bauer method.^{17,18} Susceptibility testing of *S. pneumoniae* and *S. aureus* isolates was done for penicillin, ampicillin, cefuroxime, erythromycin, cloxacillin, and cotrimoxazole (Oxoid Ltd, Basingstoke, UK). These antibiotics are commonly used in Ghana for treating bacterial infections, including those caused by *S. aureus* and *S. pneumoniae*. The antibiotic susceptibility-testing procedure employed for the bacteria isolates was as follows. On the agar plate of the test organism, a sterile loop was used to touch four to five morphologically similar colonies and inoculate 5 mL sterile saline water until the turbidity was comparable to 0.5% McFarland's standard. A loopful of the suspension was aseptically transferred to the center of a Mueller-Hinton agar plate (Oxoid Ltd), and a sterile cotton swab was used to streak the entire surface of the plate. The moisture was allowed to be absorbed for at least 15 minutes, and sterile forceps were used to apply the antibiotic discs to the surface of the agar plate while ensuring the discs were not placed less than 24 mm apart. The plates were incubated at 37°C for 18–24 hours, after which a ruler and a pair of vernier calipers were used to measure the zones of inhibition around the antibiotic discs. The results were recorded. *S. aureus* NCTC

6571 and *S. pneumoniae* NCTC 10319 were used as controls for the *S. aureus* and *S. pneumoniae* isolates, respectively. The minimum cutoff point for an *S. aureus* or *S. pneumoniae* isolate to be classified as sensitive and/or resistant was based on the NCLS break-point system.

The study protocol was approved by the Ethical and Protocol Review Committees of the School of Allied Health Sciences (University of Ghana) and the University of Ghana Medical School, and consent was obtained from parents or guardians of the children recruited for the study.

Data analysis

The data collected in the study were entered into MS Excel and analyzed in STATA 7.0 (Strata Corp, College Station, TX, USA). Descriptive analysis was carried out on the study variables, and prevalence rates were reported as percentages and 95% confidence intervals. Chi-square was used to evaluate significant associations among the study variables, and *P* values of <0.05 were considered statistically significant.

Results and discussion

Epidemiological data collected in this study, including sampling, demographic information, and bacteria carriage rates, are summarized in Table 1. The mean age of subjects recruited in the *S. aureus* carriage study was 3.5 years for those with HbSS disease and 2.5 years for those without HbSS disease (control group). The carriage rates of *S. aureus* among people with HbSS disease and people without HbSS disease were 48% (95% confidence interval (CI): 34.2–61.9), and 50% (95% CI: 36.2–63.9), respectively. There was no significant difference in *S. aureus* carriage between the two populations (*P* < 0.05). The anterior nares are the most consistent site of staphylococcal colonization, and carriage

is more common in children than in adults,^{19–21} hence the use of nasal specimens from pediatric subjects in this study. The trend of *S. aureus* carriage in this study concurs with a study done in Gabon, which reported an *S. aureus* carriage rate of 46.6% among children with HbSS disease and 46.9% among children without HbSS disease.¹⁸ About 75% of people with HbSS disease are in sub-Saharan Africa,²² and evidence from our study and from that carried out in Gabon⁸ indicates that sickling status is not a risk factor for *S. aureus* carriage. In the developed world, there are hardly any data on carriage of *S. aureus* among people with HbSS disease, though several studies have investigated carriage of the organism in the general population in some of these countries.^{23–25} In the USA, two studies carried out on children younger than 7 years reported *S. aureus* carriage rates of 9%²³ and 14%.²⁴ In Portugal, a study reported *S. aureus* carriage of 17% among children younger than 6 years.²⁵ These prevalence rates most probably apply to children with HbSS disease in the respective countries, as our data confirm that sickling status is not a risk factor for carriage of *S. aureus*. Thus, it is likely that *S. aureus* carriage among children with HbSS disease in sub-Saharan African countries such as Ghana is significantly higher than among their counterparts in the developed world, such as in the USA.

The mean age of subjects recruited in the *S. pneumoniae* carriage study was 8 years, and the carriage rate of *S. pneumoniae* was 10% (95% CI: 3.9–16.1). Carriage rates of *S. pneumoniae* among children with HbSS disease reported in other countries were 33% in Uganda,⁷ 14% in Gabon,⁸ 13% in the USA,²⁶ and 21% in the UK.²⁷ *S. pneumoniae* carriage among subjects with HbSS disease in the current study (10%) is similar to a carriage rate of 16% previously reported among HbSS disease children in Ghana,⁶ but significantly lower than most of the carriage rates (>40%) that have been reported generally among children in Ghana.^{28,29} The relatively lower carriage rate of *S. pneumoniae* among pediatric subjects with HbSS disease in Ghana, which has also been reported in Uganda⁷ and in some western countries such as the USA,²⁰ is probably due to the routine prophylactic use of penicillin among people with HbSS disease. Contrary to the disparity of *S. pneumoniae* carriage between people with and without HbSS disease in Ghana (as described above), we did not observe a relatively lower carriage rate of *S. aureus* among people with HbSS disease, probably because unlike *S. pneumoniae*, *S. aureus* is highly resistant to penicillin because of its production of beta-lactamase.²¹

As shown in Figure 1, the trend of resistance of the *S. aureus* isolates from pediatric subjects with HbSS

Table 1 Features of *Staphylococcus aureus* and *Streptococcus pneumoniae* carriage studies

Parameter	<i>S. aureus</i> study	<i>S. pneumoniae</i> study
Type of specimen used	Nasal swabs	Nasopharyngeal swabs
Number HbSS+ children	50	92
Number HbSS– children (control group)	50	No subjects recruited
Gender distribution	58 males, 34 females	54 males, 46 females
Mean age of subjects	3 years ^a	8 years
Carriage among HbSS+ children	48%	10%
Carriage among HbSS– children	50%	n/a

Note: ^aMean ages of HbSS+ children and HbSS– children (control group) were 3.5 years and 2.5 years, respectively.

Abbreviation: HbSS, homozygous SS disease.

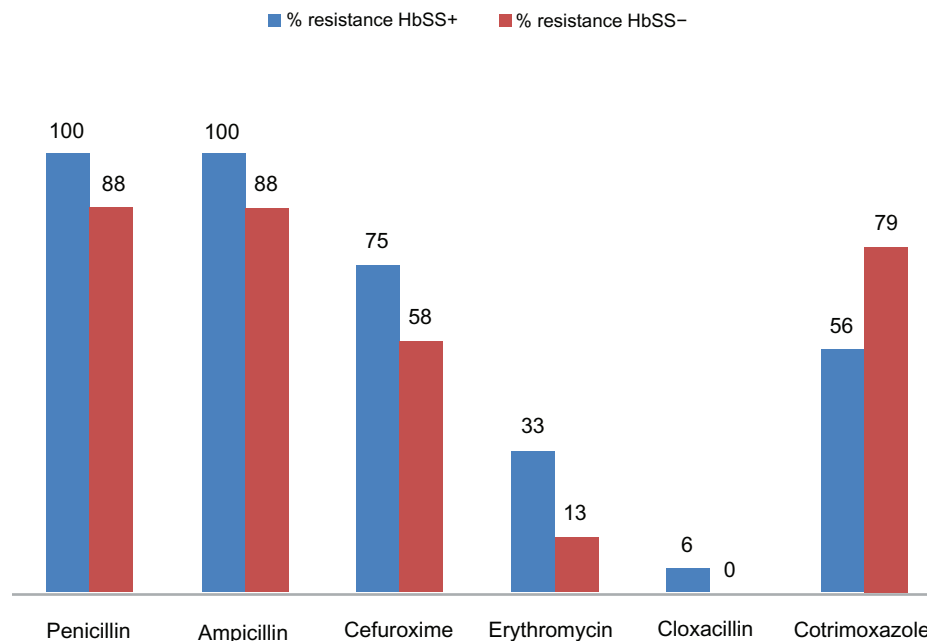


Figure 1 Antibiotic resistance of *Staphylococcus aureus* isolated from HbSS+ and HbSS- children.

Notes: The pattern of resistance of *S. aureus* isolates from HbSS+ and HbSS- children were similar and did not show any significant differences at $P < 0.05$. For both HbSS+ and HbSS- children, resistance was highest for the penicillin/ampicillin and lowest for cloxacillin.

Abbreviation: HbSS, homozygous SS disease.

disease and those without HbSS disease were similar and did not show any significant differences for all six of the antibiotics tested. By comparison, a study among Gabonese children did not show significant differences in antibiotic resistance between subjects with HbSS disease and those without HbSS disease for various encapsulated organisms, including *S. aureus*, *S. pneumoniae*, and *Haemophilus influenzae*.⁸ By contrast, a Ugandan study showed that *S. pneumoniae* carried by people with HbSS disease were highly resistant to penicillin and cotrimoxazole, compared to people without HbSS disease.⁷ This shows that the relationship between antibiotic resistance and people with HbSS disease may vary according to geographical setting, which is likely to be related to the epidemiology of antibiotic usage in a particular geographic setting. In Ghana, there is a high rate of prescriptions for antibiotics for people with HbSS disease, which may be offset by a high rate of irrational use of antibiotics, that is, through self-medication, in the general Ghanaian community.^{30,31} This may explain the absence of differences in resistance rates between *S. aureus* isolates from people with HbSS disease and those without HbSS disease in this study. In the USA, where self-medication is relatively uncommon and antibiotics such as penicillin are routinely used as prophylaxis for HbSS disease, several studies showed that *S. pneumoniae* isolated from people with HbSS disease had a significantly higher prevalence of penicillin resis-

tance, compared to isolates from people without HbSS disease.^{32,33}

The antibiotic-resistance pattern of *S. pneumoniae* isolated from pediatric subjects with HbSS disease is reported in Figure 1, and shows a very low prevalence of resistance for most of the antibiotics tested. The antibiotic-susceptibility pattern for the *S. pneumoniae* isolates is similar to that reported by a recent pneumococcal carriage study carried out generally among children in the study area.²⁹ Of interest is penicillin resistance, as the antibiotic is commonly used in pneumococcal prophylaxis among people with HbSS in Ghana. While in this study pneumococcal penicillin resistance among people with HbSS was nil, a similar study found a prevalence rate of 40% in another region of Ghana,⁶ suggesting wide variations in pneumococcal penicillin resistance in different parts of the country. The prevalence of penicillin-resistant *S. pneumoniae* among people with HbSS disease reported recently in other sub-Saharan African countries is 100% in Uganda⁷ and 30% in Gabon.⁸ These data further suggest wide variations in pneumococcal penicillin resistance in sub-Saharan Africa with respect to people with HbSS disease and to the potential for such patients to carry extremely high levels of penicillin resistance. Though we did not observe pneumococcal penicillin resistance, we predict a significant increase among people with HbSS disease in Ghana, similar to the high rates reported in other sub-Saharan African countries, due to the similarity in distribution of

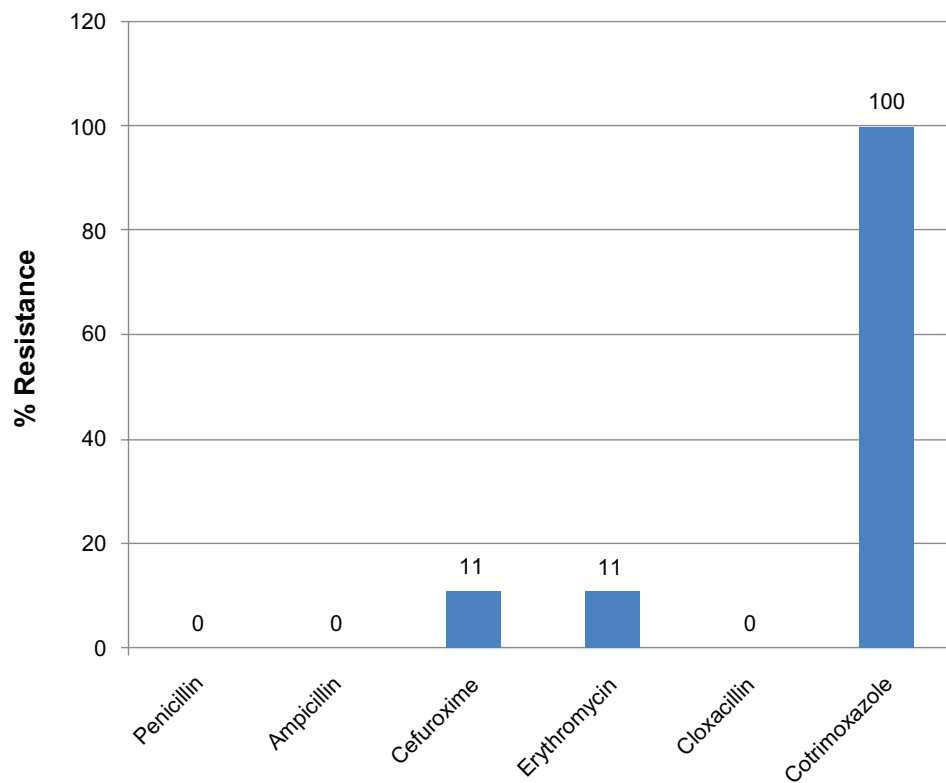


Figure 2 Antibiotic % resistance of *Streptococcus pneumoniae* isolated from HbSS+ children.

Note: Low levels of percentage resistance (0%–11%) were observed for the various antibiotics tested except cotrimoxazole, which showed an extremely high prevalence of % resistance (100%).

Abbreviation: HbSS, homozygous SS disease.

antibiotic-resistance risk factors in sub-Saharan Africa.³⁴ The increasing trend of penicillin-resistant *S. pneumoniae* strains in relation to people with HbSS disease in sub-Saharan Africa is a cause for concern, as this is likely to reduce the prophylactic ability of the antibiotic among such patients. Fortunately, *S. pneumoniae* conjugate vaccines that have proven effective in children are currently available for use in both the developed and developing world. An excellent feature of such conjugate vaccines is their ability to eliminate carriage of *S. pneumoniae* and hence reduce invasive pneumococcal disease.³⁵ The vaccines, however, do not offer a complete solution to the problem of invasive pneumococcal disease among children, as they cover only 7–13 serotypes of the 94 known pneumococcal serotypes.³⁵ It is worth noting that pneumococcal resistance to cotrimoxazole has reached its peak in Ghana, and the antibiotic is most probably useless for treating any pneumococcal infections in the country.

In Ghana, there are no surveillance data on microbial diseases in people with HbSS disease. However, there are some data from a few African countries that depict an interesting isolation pattern of causative agents. In a Ugandan study involving 155 HbSS patients, the important etio-

logical agents were *S. aureus* (60%), *H. influenzae* (19%) and *Staphylococcus epidermidis* (9%).⁹ A Kenyan study involving 108 children with HbSS disease identified the most common causes of bacteraemia as *S. pneumoniae* (41%), non-typhi *Salmonella* species (18%), and *H. influenzae* type b (12%).³⁶ In Nigeria, five studies^{10–12,37,38} have investigated bacteraemia among 57 to 304 children with HbSS disease: the most prevalent etiological agent in these studies varied, and included *S. aureus* (44%), *S. pneumoniae* (40%), *Salmonella* spp. (26%), and *Klebsiella* spp. (which had prevalence rates of 32% and 26% in two different studies). *S. pneumoniae* is probably regarded as the most important cause of invasive bacterial disease among people with HbSS disease, as shown by the institution of penicillin prophylaxis and vaccination for people with HbSS disease. However, the above data from sub-Saharan Africa indicate that other bacterial agents, especially *S. aureus*, may supersede *S. pneumoniae* as invasive diseases among people with HbSS, which partly agrees with our data showing a five-fold higher carriage rate of *S. aureus* (48%) compared to *S. pneumoniae* (10%). It is important to put the findings of the current study in the context of pneumococcal vaccination, which is currently

underway in many African countries. *S. pneumoniae* carriage is known to be inversely associated with *S. aureus* carriage in children,³⁹ which we could not investigate in our sample, as the *S. aureus* and *S. pneumoniae* carriage studies were carried out among different study subjects. This implies that massive pneumococcal vaccination underway in sub-Saharan Africa may further cause an upward shift in *S. aureus* carriage, and consequently, an increase in the burden of *S. aureus* diseases, which would be disturbing, given the emergence of methicillin-resistant *S. aureus*.^{40,41} Such an evolutionary process is expected to cause a greater burden among HbSS patients who are more susceptible to bacterial infections. In view of this, there is the need for further antibiotic susceptibility-testing studies on *S. aureus* in sub-Saharan Africa to improve empirical antibiotic treatment of *S. aureus* diseases among HbSS patients in the region.

Conclusion

This study provides some of the first data on carriage of *S. aureus* among people with HbSS disease in sub-Saharan Africa, and it indicates that sickling status is not a risk factor for carriage of *S. aureus*. The relatively high carriage of *S. aureus* among people with HbSS disease in sub-Saharan African countries such as Ghana may explain the predominance of the organism in invasive disease among people with HbSS disease in sub-Saharan Africa. Antibiotic-resistance patterns of *S. aureus* carried by people with HbSS disease and people without HbSS disease in Ghana are similar, and the *S. aureus* resistance rates are high. Carriage of antibiotic-resistant *S. pneumoniae* among the children with HbSS disease investigated in this study is quite low; however, there is a high potential for rapid increase in resistance, as observed in some sub-Saharan African areas.

The main limitations of this study are that we did not have a control arm for the carriage study on *S. pneumoniae*. Our data are preliminary, and do not cover aspects of antibiotic exposure or subtyping/serotyping of the *S. aureus* and *S. pneumoniae* that were used for investigating antibiotic resistance. A further study on the subject in these areas is desirable.

Disclosure

The authors report no conflicts of interest in this work.

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