







Nasopharyngeal Carriage and Antibiogram of Pneumococcal and Other Bacterial Pathogens from Children with Sickle Cell Disease in Tanzania

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Background: Bacterial infections contribute significantly to morbidity and mortality in sickle cell disease (SCD) patients, particularly children under five years of age. In Tanzania, prophylaxis against pneumococcal infection among children with SCD advocates the use of both oral penicillin V (PV) and pneumococcal vaccines (PNV). Therefore, this study aimed to investigate nasopharyngeal carriage and antibiogram of *Streptococcal pneumoniae* (*S. pneumoniae*) and *Staphylococcus aureus* (*S. aureus*) in children with SCD in Tanzania.

Methods: This cross-sectional study was undertaken at the two Sickle Pan-African Research Consortium (SPARCO) study sites in Dar es salaam, Tanzania. The study was conducted for six months and enrolled children with SCD between the ages of 6 to 59-months. A semi-structured questionnaire was used to collect patient data. Nasopharyngeal swabs were collected from all participants and cultured for *Streptococcal pneumoniae* and other bacterial isolates. Antimicrobial susceptibility tests of the isolates were done using the disc diffusion method.

Results: Out of 204 participants, the overall prevalence of bacterial carriage was 53.4%, with *S. aureus* (23.5%), coagulase-negative Staphylococci (CoNS) (23%) and *S. pneumoniae* (7.8%) being commonly isolated. In antibiotic susceptibility testing, *S. aureus* isolates were most resistant to penicillin (81.8%), whereas 81.3% of *S. pneumoniae* isolates were resistant to co-trimoxazole. The least antimicrobial resistance was observed for chloramphenicol for both *S. aureus* and *S. pneumoniae* isolates (6.3% versus 0%). The proportion of multi-drug resistance (MDR) was 66.7% for *S. aureus* isolates and 25% for *S. pneumoniae* isolates.

Conclusion: There are substantially high nasopharyngeal carriage pathogenic bacteria in children with SCD in Dar es Salaam, Tanzania. The presence of MDR strains to the commonly used antibiotics suggests the need to reconsider optimizing antimicrobial prophylaxis in children with SCD and advocacy on pneumococcal vaccines.

Keywords: pneumococcal carriage, bacterial pathogens, pneumococcal prophylaxis, antimicrobial resistance

Introduction

Sickle cell disease (SCD) is a group of inherited red blood cell disorders in which hemoglobin is structurally abnormal, resulting in the episodic formation of sickle-shaped red blood cells and a wide range of clinical manifestations.¹ The World Health Organization (WHO) estimates that 300,000 children are born with sickle cell disease (SCD) each year, 75% of whom are in sub-Saharan Africa.^{2,3} In Tanzania, it is estimated that there are between 8000 and 11,000 children born with SCD annually, making this the third country with the highest number of SCD-related births in the world.⁴ The

described prevalence of sickle cell trait (SCT) in Tanzania is 13%,⁵ with the highest prevalence range of 16.6% to 22.5% in northwestern Tanzania, whereas the prevalence of SCD range from 0.5% to 1.5%.⁶

Bacterial infections are the primary cause of morbidity and mortality among children. In Tanzania, pneumococcal disease resulted in more than one out of every five deaths in children younger than five years of age.⁷ Functional asplenia, negatively altered antibody production, poor opsonization, and decreased phagocytosis are among the factors that increase the risk of bacterial infections among patients with SCD.¹ Children with SCD are 600 times more likely to develop the invasive disease with *Streptococcus pneumoniae* (*S. pneumoniae*) than their age-and-sex matched controls.⁸ Therefore, the Tanzania standard treatment guideline recommends using pneumococcal prophylaxis, especially in patients with SCD.⁹ The prophylaxis includes using oral phenoxymethyl penicillin/penicillin V (PV) for children up to age five years and immunization against *S. pneumoniae* using pneumococcal conjugate vaccine (PCV-13). Moreover, PCV-13 has been incorporated into the childhood vaccination program in Tanzania since 2012. The program prescribes that all children are vaccinated starting from two months of age regardless of sickle cell status. Furthermore, the pneumococcal polysaccharide vaccine (PPSV-23) is recommended for children with SCD at two years and after every five years of life.⁹ The use of pneumococcal prophylaxis has been very effective, with a significant reduction of the incidence of invasive pneumococcal disease by 90.8% among children below two years and by 93.4% among children below five years living with SCD.¹⁰

Despite the evidence of the effectiveness of pneumococcal prophylaxis, studies conducted among patients with SCD have reported nasopharyngeal carriage of *S. pneumoniae*.^{11–16} Besides *S. pneumoniae*, high prevalence of *Staphylococcus aureus* (*S. aureus*), *Hemophilus influenza*, non-Typhi *Salmonella* species, *Klebsiella pneumoniae*, and *Escherichia coli* carriage have also been reported.^{17,18} This could be due to widespread overuse of antibiotics, the spread of resistant strains, poor compliance to prophylaxis uses, and lack of vaccines available to protect against all strains of pneumococcus.

While Tanzania is among the highest affected region with SCD, there is still a scarcity of data regarding the spectrum of bacterial carriage among patients with SCD and the appropriate antibacterial susceptibility profiles that can optimally guide these patients' proper and efficient management. Therefore, this study aimed to investigate nasopharyngeal carriage and antibiogram of *S. pneumoniae* and *S. aureus* in children with SCD in Tanzania.

Methodology

Study Design and Site

This was a cross-sectional study involving children with SCD attending sickle cell clinics from February to June 2021. The study was conducted at the Sickle Pan-African Research Consortium (SPARCO) study sites in Dar es Salaam, Tanzania. The study sites were Temeke Regional Referral Hospital and Muhimbili National Hospital. The selected study sites conduct routine sickle cell clinics, have an SCD database that has enrolled more than 400 confirmed children with SCD under five years of age and have trained health care personnel who manage patients with SCD.

Study Population

Pneumococcal prophylaxis in children with SCD includes the use of oral PV twice daily for all children with SCD until five years of age and immunization against pneumococcal infection using PCV-13 and PPSV-23. Therefore, based on the criteria guiding pneumococcal prophylaxis, the study population constituted of children with SCD aged between 6 to 59-months. The exclusion criteria were children receiving any other antibiotics during the study period and those contra-indicated to use penicillins.

Sample Size and Sampling Technique

The sample size was calculated based on the cross-sectional study design whereby prevalence (P) was the pneumococcal nasopharynx carriage among children with SCD, which was reported to be 15.3%.¹³ Therefore, a total of 204 children with SCD were enrolled. The consecutive sampling technique was used to enroll participants within the specified study period.

Data Collection

A semi-structured questionnaire was constructed by the study team and used to collect study variables such as socio-demographic characteristics (age, sex, daycare attendance), clinical data (flu, cough, difficulty in breathing, fever, and any other symptom), use of pneumococcal prophylaxis (PV and PNV), previous use of antibiotics within three months prior the participant's enrollment and laboratory data (microorganism isolated and *S. pneumoniae* antimicrobial susceptibility profile). The questions in the questionnaire were designed to answer the study objectives and the tool was pretested before the enrollment of study participants. The investigators and research assistants administered the questionnaire to the parent/guardian. The records of PV and PNV administration were verified using the antenatal clinic cards and sickle cell passports. The patients' files were used to check for clinical data and previous use of antibiotics.

Nasopharyngeal Swab Collection

The sample was collected from the nasopharynx of all enrolled children by trained personnel using a using nylon-tipped pediatrics size nasopharyngeal swab (Copan diagnostics, Murrieta, CA). Each swab was immersed immediately into a test tube containing Amies[®] transport medium (Oxoid, England).¹⁹ After that, the samples were transported cooled to the respective laboratories at the selected hospitals for culture, primary gram stain, identification and antibiotic sensitivity testing.

Laboratory Tests

Isolation and Identification of Pathogenic Bacteria

Isolation and identification of bacterial were done according to the Central Pathology Laboratory- Muhimbili National Hospital and Temeke Regional Hospital Laboratory protocols (unpublished data). Briefly, nasopharyngeal swabs were inoculated onto blood agar plates, chocolate, and MacConkey agar in duplicate. For blood agar and MacConkey agar plates, one plate for each media was incubated aerobically at 37°C for 18–24 hours. Whereas the remaining blood and chocolate agar plates were incubated anaerobically at 37°C in 5% CO₂ for 18–24 hours. Identification of bacteria was based on the standard microbiological techniques which included gram staining, catalase test, coagulase test reactions and optochin and bacitracin sensitivity testing.^{19–21}

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for *S. pneumoniae* and *S. aureus* was done by using the Kirby Bauer disc diffusion method according to criteria set by Clinical Laboratory and Standard Institute (CLSI) 31st edition 2020. For *S. pneumoniae*, isolated colonies were inoculated on sheep blood agar, incubated in 5% CO₂ for 16 to 18 hours at 37°C and *S. aureus* on Mueller-Hinton agar plates and incubated in an aerobic environment for 16 to 18 hours at 37°C. For *S. aureus*, the turbidity of the inoculated organism was standardized to a 0.5 McFarland standard then uniformly swabbed over the Mueller–Hinton agar plate. The antibiotic disk was applied into the agar medium and then incubated face up at 37 °C for 16 to 18 hours. The antimicrobial discs of interest were chosen according to the prescribing patterns in local settings as shown in Table 1.

Table 1 List of Antibiotics Tested for Susceptibility

Antibiotic	<i>S. aureus</i>	<i>S. pneumoniae</i>
Cefoxitin	30 µg	
Chloramphenicol	30 µg	30 µg
Ciprofloxacin	5 µg	5 µg
Trimethoprim/Sulfamethoxazole	1.25/23.75 µg	1.25/23.75µg
Doxycycline	30 µg	30 µg
Erythromycin	15 µg	15 µg
Gentamycin	10 µg	10 µg
Penicillin G	30 units	1 µg
Oxacillin	–	1 µg

Whereas oxacillin was tested for *S. pneumoniae*, cefoxitin was tested as a substitute for oxacillin according to the CLSI guidelines as oxacillin disk was not reliable for *S. aureus* testing. *S. aureus* isolates that displayed resistance to cefoxitin (zones of inhibition less than 22 mm), were phenotypically identified as methicillin resistance *S. aureus* (MRSA). Resistant and intermediate isolates were all referred to as non-susceptible. MDR was defined as non-susceptibility to three or more classes of antimicrobial agents including the β -lactams (ie, penicillin).^{22,23}

Data Analysis

Data were entered into MS Excel and imported into the Statistical package for social scientist ver. 23 (IBM SPSS Statistics) for analysis. Descriptive analysis including computation of arithmetic means, frequencies and percentages were presented for the study variables. The primary outcome (dependent variable) was the prevalence of pathogenic bacterial carriage in children with SCD. A univariate and multivariable logistic regression model was performed to determine factors associated with pathogenic bacterial carriage and the results were presented with odds ratios, p-values and 95% confidence intervals. A p-value of <0.05 was considered statistically significant.

Ethical Consideration

The study commenced after obtaining ethical clearance from the Muhimbili University of Health and Allied Sciences (MUHAS) review board with registration MUHAS-REC-08-2020-339. Permission to conduct the study in the selected hospitals was acquired from the hospital in-charges. Signed consents were requested from parents/guardians, followed by the assent from older children. For confidentiality purposes, each participant was given a unique study identification number. The authors confirm that the study complied with the Declaration of Helsinki.

Results

Baseline Characteristics of Study Participants

Most (61.8%) of the participants were of the age >3 years old (median 44 months; range 6–59 months). More than half were males (52.9%), and majority resided in Dar es Salaam (88.0%). The rest were from Pwani, Morogoro, Kilimanjaro, Tanga, Rukwa, Ruvuma, Singida and Njombe regions. About a third (29.9%) of participants had respiratory tract symptoms and 22.1% had used other antibiotics such as Amoxicillin, Ampiclox, Cephalexin and Co-trimoxazole in the last three months prior to the study period. The demographic and clinical characteristics of the study participants are presented in [Table 2](#).

Pneumococcal prophylaxis use among children with SCD displayed in [Figure 1](#) demonstrates that all children (100%) received PCV-13 immunization and 94.6% were also using daily PV tablets.

Bacterial Isolated from the Nasopharynx of the Participants

The overall carriage prevalence of bacterial was 53.4%, with *S. aureus* and coagulase-negative Staphylococci (CoNS) as the commonest colonizers (23.5% Vs 23%), followed by *S. pneumoniae* (7.8%). The rest (2.5%) of the isolates (*Serratia species*, *Enterobacter species*, *Klebsiella species* and *Pseudomonas species*) rarely colonized the study participants. Two participants had both *S. aureus* and *S. pneumoniae* nasopharyngeal carriage. The prevalence of bacterial carriage from the nasopharynx of the participants is presented in [Figure 2](#).

Risk Factors for Colonization of Bacterial Pathogens Among Children with SCD

The results of the logistic regression analysis indicated that children with SCD residing from regions outside Dar es salaam had a high prevalence of nasopharyngeal bacterial pathogens carriage (aOR = 3.24; 95% CI = 1.41–7.4). The presence of CoNS carriage was significantly associated with the lower prevalence of bacterial pathogens among children with SCD (38.9% Vs 8.5%). Participants with the absence of non-pathogenic CoNS colonization had 7.26 (95% CI 2.43–21.69) times the odds of pathogenic bacterial carriage than those who had CoNS. Details of the risk factor analysis are presented in [Table 3](#).

Table 2 Demographic and Clinical Characteristics of the Study Participants (n = 204)

Characteristics	Frequency (n)	Proportion (%)
Age (years)		
< 1 year	3	1.5
1–3 years	75	36.8
>3 years	126	61.8
Sex		
Female	96	47.1
Male	108	52.9
Region		
Dar es salaam	172	84.3
Others	32	15.7
Attend daycare		
Yes	101	50.5
No	103	49.5
Respiratory symptoms		
Yes	61	29.9
No	143	70.1
Flu		
Yes	28	13.7
No	176	86.3
Cough		
Yes	41	20.1
No	163	79.9
Fever		
Yes	23	11.3
No	181	88.7
DIB		
Yes	7	3.4
No	197	96.6
Other antibiotic use (within three months)		
Yes	45	22.1
No	159	77.9

Patterns of Antimicrobial Resistance Among *S. aureus* and *S. pneumoniae* Isolates

In the current study, penicillin was the antimicrobial to which the *S. aureus* isolates displayed the highest resistance (81.8%). A total of 42% of *S. aureus* isolates were resistant to ceftazidime (MRSA). On the other hand, *S. pneumoniae* was highly resistant to co-trimoxazole (81.3%). A quarter (25%) of *S. pneumoniae* isolates were resistant to penicillin. The lowest antimicrobial resistance was recorded with chloramphenicol with both *S. aureus* and *S. pneumoniae* (6.3% versus 0%). The proportion of MDR (resistance to more than two antibiotic classes) were 66.7% for *S. aureus* isolates and 25% for *S. pneumoniae* isolates. Details of the prevalence of antibacterial resistance of *S. aureus* and *S. pneumoniae* isolates are shown in Figure 3A and B.

Discussion

Globally, patients with SCD have many and different unmet needs both in prevention and treatment of clinical manifestations.²⁴ It is estimated that up to 90% of patients with SCD reside in low-middle income countries (LMICs), and 90% of children with SCD in LMICs die before their fifth birthday.^{25,26} Therefore, this study aimed to investigate

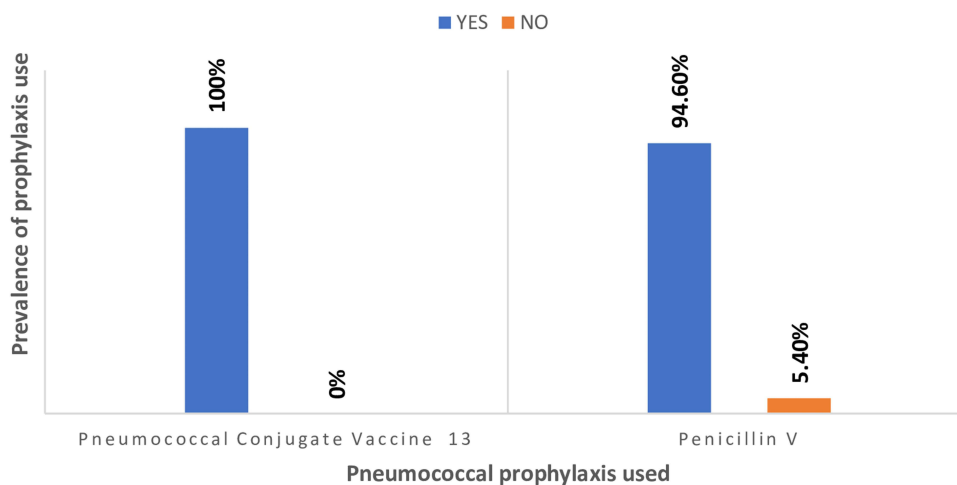


Figure 1 Pneumococcal prophylaxis use among children with SCD (n = 204).

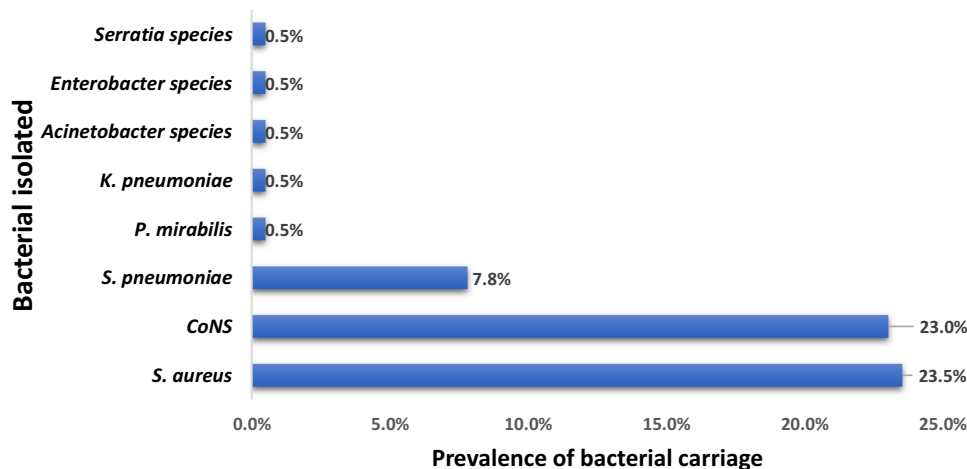


Figure 2 Prevalence of bacteria isolated from children with SCD (n = 204).

nasopharyngeal carriage and antibiogram of *S. pneumoniae* and *S. aureus* in children with SCD in Tanzania. The antibiotic resistance profile was also determined. To our knowledge, this is the first study focusing on children with SCD under five years since the incorporation of PCV-13 (pneumococcal vaccine) into the childhood vaccination program in late 2012 in Tanzania.

The overall nasopharyngeal bacterial carriage among children with SCD was 54.3%. The isolated bacterial flora belonged to both the Gram-positive and Gram-negative categories, comprising *S. aureus*, CoNS, *S. pneumoniae*, *Serratia species*, *Enterobacter species*, *Klebsiella species* and *Pseudomonas species*. Notably, while other bacteria isolates were present in low numbers, *S. aureus* and *S. pneumoniae* prevalence were 23.5% and 7.8%, respectively, underscoring the domination of *S. aureus* amongst isolated nasopharyngeal colonizers.

The human nasopharynx is the primary reservoir for *S. pneumoniae*, but not *S. aureus*. Several studies conducted post PCV-13 vaccine rollout have also reported a higher prevalence of *S. aureus* nasopharyngeal colonization in relation to *S. pneumoniae* among children with SCD.^{27,28} Therefore, the significantly higher carriage of *S. aureus* relative to *S. pneumoniae* among the participants of our study, as well as the presence of the other nasopharyngeal colonizers in low numbers, suggest the possible modification of the nasopharyngeal microbiota which can be a result of the use of pneumococcal prophylaxis in children with SCD.²⁹ This raises legitimate concerns, as *S. aureus*, although a commensal,

Table 3 Determinants of Bacterial Pathogens Carriage Among Children with SCD (n=204)

Variable	Proportion	cOR	95% CI	p-value	aOR	95% CI	p-value
Age (months)	65/204 (31.9%)	0.99	0.98–1.02	0.834			
Sex							
Female	32/64 (33.3%)	1.14	0.63–2.05	0.671			
Male	33/75 (30.6%)	Reference					
Region							
Others	17/32 (53.1%)	2.93	1.36–6.32	0.006	3.24	1.41–7.44	0.006
Dar es salaam	48/172 (27.9%)	Reference					
Daycare							
No	31/103 (30.1%)	0.85	0.47–1.53	0.585			
Yes	34/101 (33.7%)	Reference					
Respiratory symptoms							
No	46/143 (32.1%)	1.05	0.55–1.99	0.886			
Yes	19/61 (31.1%)	Reference					
Flu							
No	57/173 (32.9%)	1.12	0.48–2.88	0.688			
Yes	8/28 (28.6%)	Reference					
Cough							
No	53/163 (32.5%)	1.16	0.55–2.46	0.690			
Yes	12/41 (29.3%)	Reference					
Fever							
No	57/181 (31.5%)	0.86	0.35–2.15	0.750			
Yes	8/23 (34.8%)	Reference					
DIB							
No	63/197 (32.0%)	1.18	0.22–6.23	0.849			
Yes	2/7 (28.6%)	Reference					
Penicillin V							
No	4/11 (36.4%)	1.24	0.35–4.38	0.742			
Yes	61/193 (31.6%)	Reference					
Other antibiotics							
No	56/159 (35.2%)	2.18	0.98–4.84	0.057	2.16	0.96–4.86	0.064
Yes	9/49 (20.0%)	Reference					
CoNS colonization							
No	61/157 (38.9%)	6.83	2.33–19.99	0.000	7.26	2.43–21.69	0.000
Yes	4/47 (8.5%)	Reference					

has evolved into a significant human pathogen over the years, causing mild to severe infections, including folliculitis and furunculosis, meningitis, and septicemia, pneumonia, endocarditis, and osteomyelitis.^{30,31} Therefore, its presence in the nasopharynx significantly predicts subsequent invasive infections.^{32,33}

The prevalence of *S. aureus* within this study population was lower than reported in similar studies conducted in Ghana and Brazil where higher nasopharyngeal prevalence of 57.9% and 44.8%, respectively, have been documented.^{27,28} The differences could be due to the nature of the study population, pneumococcal prophylaxis uses between the two populations, and the antimicrobial resistance pattern differences.

S. pneumoniae nasopharyngeal carriage is a precursor for invasive pneumococcal disease; as a result, reduction or prevention of nasopharyngeal *S. pneumoniae* carriage may reduce the transmission of pneumococci. In this study, all enrolled children had received three doses of PCV-13 and over 90% were also using PV tablets for prophylaxis. Despite the recommendation on the use of PPSV-23 for children with SCD from two years old, none of SCD children were reported to have received this vaccine in this study. The prevalence of *S. pneumoniae* carriage in this population was lower compared to that previously reported in Tanzania among children without SCD (31%),²² and those reported in children with SCD in other African countries such as Gabon (13.8%), Uganda (33%) and Ghana (39.1%).^{14–16} The lower

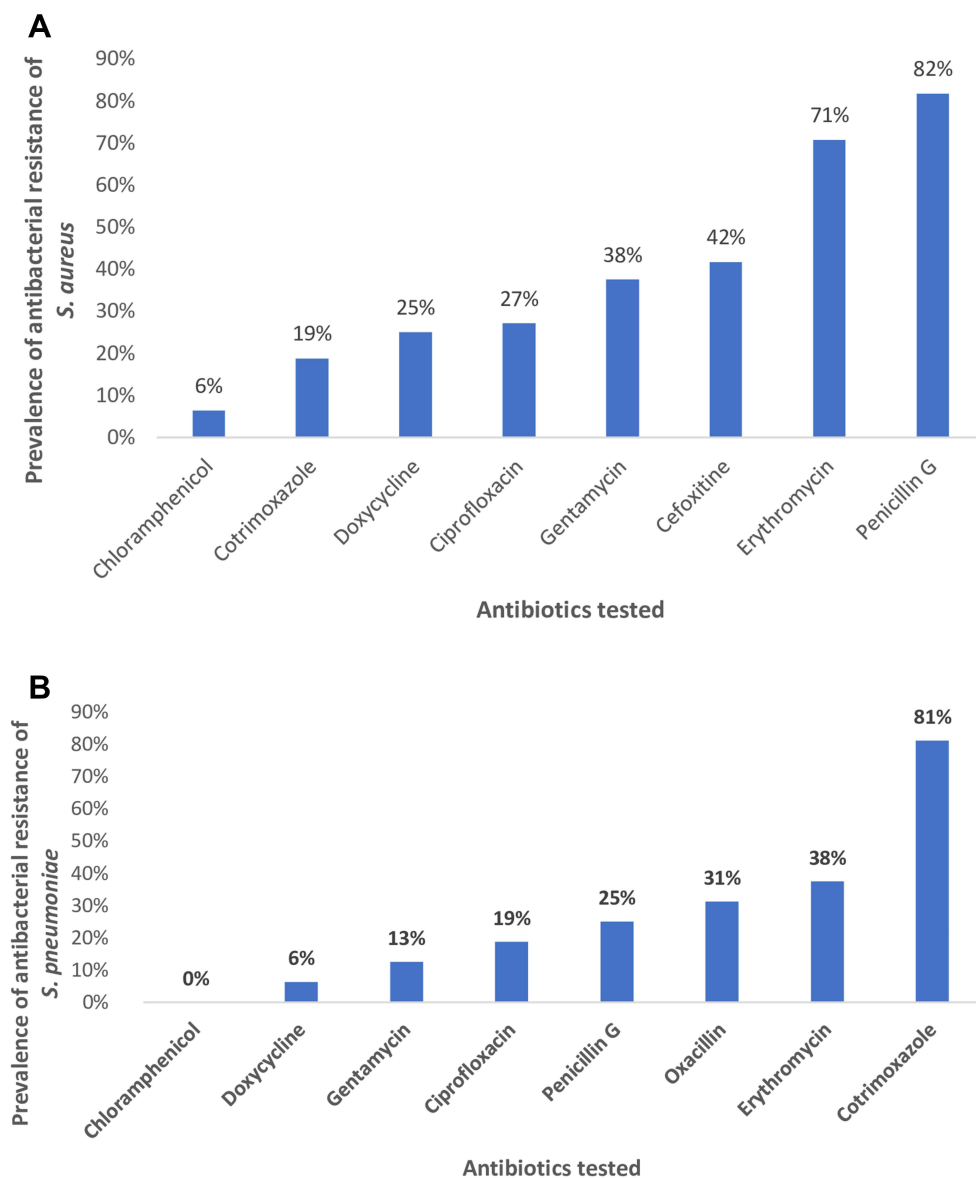


Figure 3 (A) Antibacterial resistance prevalence of *S. aureus* isolates (n = 48). (B) Antibacterial resistance prevalence of *S. pneumoniae* isolates (n = 16).

prevalence observed in this study could be due to a high number of participants using pneumococcal prophylaxis compared to the previous studies. Several studies have proven that PCV-13 and PV prophylaxis significantly reduce pneumococcal carriage.^{14,34,35}

One of the protective factors against nasopharyngeal pathogenic bacterial carriage observed among children in this study was the presence of CoNS. These are generally non-pathogenic commensals of humans and other animals and are antagonistic to *S. aureus*. Recent studies have described several interactions between CoNS and *S. aureus* that share similar host niches.^{36–38} CoNS strains have been reported to prevent *S. aureus* colonization,³⁹ an observation made in this study.

Children residing in regions outside Dar es salaam had a high bacterial carriage. This could be hypothesized by the fact that SPARCO clinics providing services to SCD patients are well established in Dar es Salaam compared to other regions in Tanzania and offer care and training of not only to health care professionals but also to caregivers and patients with SCD. Those living outside Dar es salaam incur more cost to attend the clinic, resulting in frequent interruptions in clinic attendance, contributing to inadequate intake of pneumococcal prophylaxis provided at the clinic, leading to poor

related health outcomes. Previous studies have also reported age as the risk factor whereby the colonization rate rises from birth until it peaks around 1–2 years, followed by the decline,^{40,41} Other determinants of bacterial carriage among children with SCD reported in previous studies include the presence of respiratory symptoms, daycare attendance, and self-medication.^{16,42–45} Disparities in these determinants show dissimilarity in the risk factors that could result in the bacterial carriage from one population to another. Moreover, the variations in the uptake of interventions required to prevent infections within the population may also change risk factors. Hence, these studies need to be conducted in various settings so that the findings can help design interventions suitable for that population.

Monitoring antimicrobial resistance among children with SCD using penicillin prophylaxis is critical for planning a successful therapeutic guideline and preventing further emergence of antibiotic resistance. The highest antibiotic resistance rate exhibited by *S. aureus* was recorded against penicillin, which was expected. Penicillin is routinely used among SCD individuals for prophylactic purposes against *S. pneumoniae* infections. Moreover, other recent *S. aureus* studies conducted in children with SCD have reported similar resistance rates against it.^{16,42} In this study, resistance to penicillin was recorded in 25% of pneumococcal isolates, which is lower than that reported among SCD population in Ghana (37.4%) and Uganda (100%).^{14,16} The prevalence of resistant strains in the population may be directly linked to the high usage of antibiotics in Tanzania, where antibiotics are more prescribed in children than adults.⁴⁶ Penicillins have been on the market for a very long time; this coupled with the high rates of irrational antibiotics use in the country,^{47,48} contributes significantly to the increasing burden of drug resistance to this group of antibiotics. In contrast to our findings, high resistance rates to penicillin (ranging from 70.6% to 100%) have been reported in other several studies conducted in general population in Tanzania.^{49–51} This threatens the effectiveness of PV for prophylaxis of pneumococcal infections among children with SCD especially in African countries.

A high rate of co-trimoxazole-resistant *S. pneumoniae* colonizing the nasopharynx was also observed in this study. Co-trimoxazole is widely used in resource-constrained countries, including Tanzania. A previous study indicated that co-trimoxazole use increases the risk of carriage of co-trimoxazole-resistant *S. pneumoniae*.⁵² Pneumococcal resistance to co-trimoxazole among children with SCD has been reported in other studies conducted in Africa.^{14,16} The high rate of co-trimoxazole-resistant *S. pneumoniae* colonizing the nasopharynx observed in this study is also in line with previous findings reported from HIV-infected populations in Tanzania.^{49,53}

In this study, a total of 42% of isolates were phenotypically categorized as MRSA. Carriage of MRSA has also been reported in previous studies conducted in SCD patients.^{27,42} MRSA can cause a range of difficult-to-treat infections such as osteomyelitis, meningitis, pneumonia, lung abscess, and empyema.⁵⁴ Resistance to erythromycin, ciprofloxacin, cefoxitin and gentamicin have been previously reported in *S. aureus* and *S. pneumoniae*.^{16,27,42,55} The low rate of chloramphenicol resistance in both staphylococcal and pneumococcal strains, although comforting, lacks clinical significance, as the drug is rarely used in clinical practice.

The 66.7% proportion of MDR observed in *S. aureus* isolated from this study appears to be within the range of those reported in other previous studies (62.3–100%).^{42,56} The presence of MDR strains may be due to the irrational use of antimicrobials in the community whereby the antibiotics are sold without prescriptions and extensively used in farming. The prevalence of pneumococcal MDR observed (25%) is lower than that reported in children with SCD in Ghana (34.3%). However, the reported pneumococcal MDR prevalence, is similar to the prevalence recently reported among HIV-infected patients (26.3%)⁴⁹ and in healthy children (23%) in Tanzania.²² This is very disturbing as patients with SCD have a relatively higher risk of pneumococcal infections when compared to the general population.

Conclusion

This study concludes that bacterial carriage is common among children with SCD in Tanzania, whereby *S. aureus* is currently dominating. *S. aureus* and *S. pneumoniae* strains carried by the children with SCD showed resistance to commonly used antibiotics, with MRSA and MDR being significantly high. The isolated pathogens in this study have been implicated in various invasive infections in patients with SCD. The high levels of bacterial resistance have important implications for preventing and treating such infections in the population. Since this study enrolled only children with SCD attending sickle cell clinics under SPARCO Tanzania, we recommend that further studies be conducted countrywide to determine the bacterial carriage and antibiotics resistance pattern required for decision-

making. We also recommend continuous intensification of public health education against irrational antibiotic use. There is also a need to monitor patients with SCD for invasive bacterial diseases due to *S. aureus*.

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Disclosure

The authors report no conflicts of interest in this work.

References

- Booth C, Inusa B, Obaro SK. Infection in sickle cell disease: a review. *Int J Infect Dis*. 2010;14(1):e2–e12.
- Silva-Nunes MD, Ferreira MU. Clinical spectrum of uncomplicated malaria in semi-immune Amazonians: beyond the “symptomatic” vs “asymptomatic” dichotomy. *Mem Inst Oswaldo Cruz*. 2007;102(3):341–348.
- Roucher C, Rogier C, Dieye-Ba F, et al. Changing malaria epidemiology and diagnostic criteria for *Plasmodium falciparum* clinical malaria. *PLoS One*. 2012;7(9):e46188.
- Piel FB, Patil AP, Howes RE, et al. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet*. 2013;381(9861):142–151.
- Makani J, Cox SE, Soka D, et al. Mortality in sickle cell anemia in Africa: a Prospective Cohort Study in Tanzania. *PLoS One*. 2011;6:e14699.
- Ambrose EE, Makani J, Chami N, et al. High birth prevalence of sickle cell disease in Northwestern Tanzania. *Pediatr Blood Cancer*. 2018;65(1):584. doi:10.1002/pbc.26735
- World Health Organization (WHO). 23-valent pneumococcal polysaccharide vaccine. *Wkly Epidemiol Rec*. 2008;83(42):373–384.
- Overturf G, Powars D, Baraff L. Bacterial meningitis and septicemia in sickle cell disease. *Am J Diseases Childhood*. 1977;131:784–787.
- Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania Mainland], Ministry of Health (MoH) [Zanzibar], National Bureau of Statistics (NBS), Office of the Chief Government Statistician (OCGS), and ICF. *Tanzania Malaria Indicator Survey 2017*. Dar-es-Salaam, Tanzania, and Rockville, Maryland, USA: MoHCDGEC, MoH, NBS, OCGS, and ICF; 2017.
- Halasa NB, Shankar SM, Talbot TR, et al. Incidence of invasive pneumococcal disease among individuals with sickle cell disease before and after the introduction of the pneumococcal conjugate vaccine. *Clin Infect Dis*. 2007;44:1428–1433.
- Fonseca PB, Braga JA, Machado AM, et al. Nasopharyngeal colonization by *Streptococcus pneumoniae* in children with sickle cell disease receiving prophylactic penicillin. *J Pediatr (Rio J)*. 2005;81:149–154.
- Alexander E, Telfer P, Rashid H, et al. Nasopharyngeal carriage rate of *Streptococcus pneumoniae* in children with sickle cell disease before and after the introduction of heptavalent pneumococcal conjugate vaccine. *J Infect Public Health*. 2008;1(1):40–44.
- Donkor ES, Newman MJ, Oliver-Commey J, et al. Invasive disease and paediatric carriage of *Streptococcus pneumoniae* in Ghana. *Scand J Infect Dis*. 2010;42(4):254–259.
- Kateete DP, Kajumbula H, Kaddu-Mulindwa DH, et al. Nasopharyngeal carriage rate of *Streptococcus pneumoniae* in Ugandan children with sickle cell disease. *BMC Res Notes*. 2012;5:28.
- Schaumburg F, Biallas B, Ngoune Feugap E, et al. Carriage of encapsulated bacteria in Gabonese children with sickle cell anaemia. *Clin Microbiol Infect*. 2013;19(3):235–241.
- Dayie NTKD, Tetteh-Ocloo G, Labi AK, et al. Pneumococcal carriage among sickle cell disease patients in Accra, Ghana: risk factors, serotypes and antibiotic resistance. *PLoS One*. 2018;13(11):e0206728.
- Makani J, Mgaya J, Balandya E, et al. Bacteraemia in sickle cell anaemia is associated with low haemoglobin: a report of 890 admissions to a tertiary hospital in Tanzania. *Br J Haematol*. 2015;171:273–276.
- Alima Yanda AN, Nansseu JRN, Mbassi Awa HD, et al. Burden and spectrum of bacterial infections among sickle cell disease children living in Cameroon. *BMC Infect Dis*. 2017;17:211.
- Carroll K, Reimer L. Microbiology and laboratory diagnosis of upper respiratory tract infections. *Clin Infect Dis*. 1996;23(3):442–448.
- Tesfaw G, Kibru G, Mekonnen D, et al. Prevalence of group A β -haemolytic *Streptococcus* among children with pharyngitis in Jimma town, Southwest Ethiopia. *Egypt J Ear Nose Throat Allied Sci*. 2015;16(1):35–40.
- Patel JB, Cockerill F, Bradford PA, et al. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement*. Clinical and Laboratory Standards Institute; 2015.

22. Emgård M, Msuya SE, Nyombi BM, et al. Carriage of penicillin-non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. *Int J Infect Dis.* 2019;81:156–166.
23. Finkelstein JA, Huang SS, Daniel J, et al. Antibiotic-resistant *Streptococcus pneumoniae* in the heptavalent pneumococcal conjugate vaccine era: predictors of carriage in a multicomunity sample. *Pediatrics.* 2003;112:862–869.
24. Colombatti R, Palazzi G, Masera N, et al. Italian Multicenter Study of Hydroxyurea in Sickle Cell Anemia Investigators. Hydroxyurea prescription, availability and use for children with sickle cell disease in Italy: results of a National Multicenter survey. *Pediatr Blood Cancer.* 2018;65(2):28868627.
25. McGann PT. Time to invest in sickle cell anemia as a global health priority. *Pediatrics.* 2016;137:e20160348.
26. Grosse SD, Odame I, Atrash HK, et al. Sickle cell disease in Africa: a neglected cause of early childhood mortality. *Am J Prev Med.* 2011;41(6, suppl 4):S398–S405.
27. Dayie NTKD, Sekoh DNK, Kotey FCN, et al. Nasopharyngeal Carriage of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Sickle Cell Disease (SCD) Children in the Pneumococcal Conjugate Vaccine Era. *Infect Dis Rep.* 2021;13(1):191–204.
28. Rocha LC, Carvalho MO, Nascimento VM, et al. Nasopharyngeal and Oropharyngeal Colonization by *Staphylococcus aureus* and *Streptococcus pneumoniae* and Prognostic Markers in Children with Sickle Cell Disease from the Northeast of Brazil. *Front Microbiol.* 2017;8:217.
29. Donkor ES, Foster-Nyarko E, Enweronu-Laryea CC. Relationship between antibiotic resistance and sickle cell anemia: preliminary evidence from a pediatric carriage study in Ghana. *Infect Drug Resist.* 2013;6:71–77.
30. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med.* 1998;339:520–532.
31. Todar K. *Todar's Online Textbook of Bacteriology.* Department of Bacteriology. Madison, WI, USA: University of Wisconsin-Madison; 2006.
32. Pizzutto SJ, Hare KM, Upham JW. Bronchiectasis in children: current concepts in immunology and microbiology. *Front Pediatrics.* 2017;29:123.
33. Nzenze SA. Effect of introduction of pneumococcal conjugate vaccine immunization on nasopharyngeal colonization of *Streptococcus pneumoniae* in South Africa (Doctoral dissertation) Wits Inst. *Environ D-Space.* 2015;212:386.
34. Grant LR, Hammitt LL, O'Brien SE, et al. Impact of the 13-Valent Pneumococcal Conjugate Vaccine on Pneumococcal Carriage Among American Indians. *Pediatr Infect Dis J.* 2016;35(8):907–914.
35. Anglin DL, Siegel JD, Pacini DL, et al. Effect of penicillin prophylaxis on nasopharyngeal colonization with *Streptococcus pneumoniae* in children with sickle cell anemia. *J Pediatr.* 1984;104:18–22.
36. Nakatsuji T, Chen TH, Narala S, et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med.* 2017;9:845.
37. Janek D, Zipperer A, Kulik A, et al. High Frequency and Diversity of Antimicrobial Activities Produced by Nasal *Staphylococcus* Strains against Bacterial Competitors. *PLoS Pathog.* 2016;12:e1005812.
38. Zipperer A, Konnerth MC, Laux C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. *Nature.* 2016;535:511–516.
39. Paharik AE, Parlet CP, Chung N, et al. Coagulase-negative staphylococcal strain prevents *Staphylococcus aureus* colonization and skin infection by blocking quorum sensing. *Cell Host Microbe.* 2017;22:746–756.
40. Usuf E, Bottomley C, Adegbola RA, et al. Pneumococcal Carriage in Sub-Saharan Africa—A Systematic Review. *PLoS One.* 2014;9(1):e85001.
41. Hussain M, Melegaro A, Pebody RG, et al. A longitudinal household study of *Streptococcus pneumoniae* nasopharyngeal carriage in a UK setting. *Epidemiol Infect.* 2005;133:891–898.
42. Appiah VA, Pesewu GA, Kotey FCN, et al. *Staphylococcus aureus* Nasal Colonization among Children with Sickle Cell Disease at the Children's Hospital, Accra: prevalence, Risk Factors, and Antibiotic Resistance. *Pathogens.* 2020;9(5):329.
43. Mills RO, Twum-Danso K, Owusu-Agyei S, et al. Epidemiology of pneumococcal carriage in children under five years of age in Accra, Ghana. *Infect Dis J.* 2015;47:326–331.
44. Lemma MT, Zenebe Y, Tulu B, et al. Methicillin Resistant *Staphylococcus aureus* among HIV Infected Pediatric Patients in Northwest Ethiopia: carriage Rates and Antibiotic Co-Resistance Profiles. *PLoS One.* 2015;10:e0137254.
45. Sleeman KL, Daniels L, Gardiner M, et al. Acquisition of *Streptococcus pneumoniae* and nonspecific morbidity in infants and their families: a cohort study. *J Infect Dis.* 2005;194(5):682–688.
46. Seni J, Mapunjo SG, Wittenauer R, et al. Antimicrobial use across six referral hospitals in Tanzania: a point prevalence survey. *BMJ Open.* 2020;10:e042819.
47. Ngocho JS, Horumpende PG, de Jonge MI, et al. Inappropriate treatment of community-acquired pneumonia among children under five years of age in Tanzania. *Int J Infect Dis.* 2020;93:56–61.
48. Mboya E, Sanga L, Ngocho J. Irrational use of antibiotics in the Moshi Municipality Northern Tanzania: a cross sectional study. *Pan Af Med J.* 2018;31:165.
49. Manyahi J, Moyo S, Aboud S, et al. High rate of antimicrobial resistance and multiple mutations in the dihydrofolate reductase gene among *Streptococcus pneumoniae* isolated from HIV-infected adults in a community setting in Tanzania. *J Global Antimicrobial Resistance.* 2020;22:749–753.
50. Kumburu HH, Sonda T, Mmbaga BT, et al. Patterns of infections, aetiological agents and antimicrobial resistance at a tertiary care hospital in northern Tanzania. *Trop Med Int Health.* 2017;22:454–464.
51. Moyo S, Aboud S, Kasubi M, et al. Bacteria isolated from bloodstream infections at a tertiary hospital in Dar es Salaam, Tanzania—antimicrobial resistance of isolates. *S Afr Med J.* 2010;100:835–838.
52. Karpanoja P, Nyberg ST, Bergman M, et al. Connection between trimethoprim-sulfamethoxazole uses and resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. *Antimicrob Agents Chemother.* 2008;52:2480–2485.
53. Bles P, de Mast Q, van der Gaast-de Jongh CE, et al. Antibiotic resistance of *Streptococcus pneumoniae* colonising the nasopharynx of HIV-exposed Tanzanian infants. *Trop Med Int Health.* 2015;20:1559–1563.
54. Siddiqui AH, Koirala J. *Methicillin Resistant Staphylococcus aureus*. Treasure Island (FL): StatPearls Publishing;2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK482221/>. Accessed August 1, 2022.
55. Stacevičienė I, Petraitienė S, Vaičiūnienė D, et al. Antibiotic resistance of *Streptococcus pneumoniae*, isolated from nasopharynx of preschool children with acute respiratory tract infection in Lithuania. *BMC Infect Dis.* 2016;16:216.
56. Donkor ES, Kotey FCN, Dayie NTKD, et al. Colonization of HIV-Infected Children with Methicillin-Resistant *Staphylococcus aureus*. *Pathogens.* 2019;8:35.

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