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Nasal colonization of methicillin-resistant *Staphylococcus aureus* in HIV-infected patients at the Cape Coast Teaching Hospital, Ghana

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Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be associated with outbreaks in communities (CA-MRSA) and hospitals (HA-MRSA). MRSA isolates are known to be resistant to all beta-lactam antibiotics including methicillin. Moreover, HIV-infected individuals are highly at risk of CA-MRSA due to their weaker immune system. It is therefore important to keep surveillance of the prevalence. Our study aims at determining the prevalence of *Staphylococcus aureus* and MRSA among HIV-infected participants, the bacteria's associations, and their antibiotic susceptibility patterns. A cross-sectional study was conducted and nasal swabs from 657 participants attending the HIV clinic at the Cape Coast Teaching Hospital were taken following guidelines. Confirmed *S. aureus* isolates were taken through antibiotic susceptibility tests per the Kirby–Bauer method, and isolates that were resistant to cefoxitin were considered to be MRSA. The carriage prevalence of *S. aureus* and MRSA was 44.7% and 8.2%, respectively, among the HIV-infected individuals. There was a significant association between hospitalization and MRSA colonization ($p = 0.002$), but not *S. aureus* colonization ($p = 0.266$). Significant association was also observed between age ($p = 0.001$), sex ($p = 0.0001$), and *S. aureus* colonization. Similarly, differences in age groups ($p = 0.001$), sex ($p = 0.02$), and MRSA colonization were statistically significant ($p = 0.001$). High percentage resistance was exhibited by the isolates to most of the antibiotics. However, this study did not record vancomycin resistance among the MRSA strains. The study showed high colonization of *S. aureus* and MRSA in HIV-infected patients, which was mostly associated with the age and sex of the individuals.

KEYWORDS

HIV-infected patients, susceptibility, MRSA, antibiotics, *Staphycoccus aureus*

Background

Staphylococcus aureus exists as a commensal on the surfaces of human mucosa, and it is one of the leading pathogens that cause infections such as osteomyelitis, bacteremia, endocarditis, and meningitis in humans (1). *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), has been reported to be responsible for the augmented number of global hospital- and community-acquired infections. MRSA is a resistant isolate that has acquired the staphylococcal cassette chromosome *mec* (SCC mec), which contains the *mecA* gene. The *mecA* gene encodes for the altered penicillin-binding protein 2a (PBP2a), which mediates resistance to all beta-lactam antibiotics including methicillin (2). *S. aureus* resistance to methicillin was first discovered in the United Kingdom in 1961 and in Africa in 1978 (3). Since then, hospital-related outbreaks caused by hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and community-acquired MRSA (CA-MRSA) have been reported. Popovich et al. reported a 6- to 18-fold increase in MRSA infection susceptibility compared to the general population (4). It has also been shown that HIV-infected individuals have a high risk (18-fold increase) of carrying CA-MRSA (5). In Ghana, there have been reports of *S. aureus* and MRSA carriage prevalence. Although the prevalence of *S. aureus* and MRSA carriage was found to be 13.9% and 13%, respectively, among inpatients (6), they were found to be 44.9% and 5.6%, respectively, among HIV-infected individuals (7).

Nasal colonization of MRSA among HIV patients is significant not only for predisposing the HIV patients to subsequent infections but also in playing a vital role in the transmission of MRSA to other patients and the general population (8). The emergence of resistant strains of *S. aureus* in HIV patients and the rising prevalence in the community highlight the need for worldwide epidemiological studies of this pathogen. There is limited information on the prevalence and risk factors for MRSA colonization among HIV patients in Africa with no exception in Ghana. The few studies in Ghana have been concentrated in Accra. Therefore, this study was conducted to fill the existing knowledge gap by determining the nasal colonization of MRSA and related risk factors in HIV-infected patients in the Cape Coast Teaching Hospital (CCTH).

Materials and methods

Study period and study setting

This cross-sectional study was conducted between January and May 2018 at the CCTH in Cape Coast, Central Region, Ghana. The hospital receives referrals from all districts in the region and nearby regions. The hospital has an approximate bed capacity of 400 and an annual approximate outpatient department attendance of 350,000. The participants selected for this study were HIV patients who were visiting CCTH for antiretroviral therapy.

Data and sample collection

A well-structured questionnaire combined with a review of medical records was used for collecting the sociodemographic, risk factors, and clinical characteristics of HIV-positive individuals. Age, sex, hospitalization, occupation, religion, educational level, marital status, CD4 counts, and viral load were obtained.

Nasal swabs were taken using sterile swab sticks and immediately immersed in peptone water. The samples were soon after transported on ice to the Department of Biomedical Science Microbiology laboratory for inoculation on prepared media.

Inclusion criteria

Participants selected for this study were HIV out- and inpatients who were receiving their antiretroviral therapy at the CCTH with no signs of flu nor were on antibiotic or antimicrobial agents.

Exclusion criteria

Participants who were on antibiotics or any antimicrobial agents at the time of sample taking were excluded from this study.

Ethical clearance and consent approval

The research proposal was approved by the Research and Developmental Secretariat of the CCTH (Ref: CCTH/RDS/2017/03). A consent was obtained from the participants before a nasal swab was taken from the anterior nares using previously described procedures (9).

Isolation and identification of *Staphylococcus aureus*

Nasal swabs were processed according to the method previously described by Egyir et al. (6). A sterile cotton swab was used in taking samples by rotating it five times in both anterior nares. The nasal swabs were inoculated on a selective and differential medium, mannitol salt agar (MSA) (Oxoid,

Hampshire, UK), which was then incubated at 37°C for 18–24 h. Yellow colonies on the MSA were suspected to be *S. aureus* and were therefore taken through gram staining, catalase testing, and tube coagulase testing for confirmation using ATCC 25923 strain as a control.

MRSA detection and antimicrobial susceptibility testing

Four to five bacteria colonies were inoculated into a sterile tube containing 0.5 ml of normal saline, and turbidity was adjusted to match that of 0.5 M McFarland standards in order to obtain approximately the organism number of colony-forming units (CFU)/ml. Subsequently, a sterile swab was dipped into the suspension, and the excess of the inoculum was removed by pressing it against the sides of the tube. The swab was streaked on a Mueller–Hinton agar plate and evenly spread on the medium. Following 15 min of inoculation to the Mueller–Hinton agar, antibiotics were placed on the inoculated agar and incubated at 37°C for 24 h. The following drugs and their respective concentrations were used to determine the antibiogram of the strains: ampicillin/sulbactam (20 µg), co-trimoxazole (25 µg), cephalexin (30 µg), tetracycline (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), prulifloxacin (5 µg), ofloxacin (5 µg), cloxacillin (5 µg), roxithromycin (15 µg), lincomycin (2 µg), gentamycin (10 µg), vancomycin (30 µg), mupirocin (200 µg), and cefoxitin (30 µg). Isolates that were resistant to cefoxitin were considered to be MRSA-positive as described by CLSI (10).

Data analysis

Variables from demographic and clinical data obtained from the questionnaire and laboratory data were entered into a Microsoft Excel spreadsheet and were later analyzed using SPSS version 22 for Windows. The chi-square test (χ^2) was used to determine the association between the categorical variables, and $P < 0.05$ was considered to be statistically significant.

Results

Sociodemographic distribution

A total of 657 participants were enrolled: 205 (31.1%) men and 452 (68.9%) women whose ages ranged from 11 to 75 years. Majority of the participants (228, 34.7%) were between the ages of 40 and 49 years. Most of the participants were single (345, 52.5%) and Christians (447, 68%). Regarding their level of education, 231 (35.2%) of the participants were uneducated, 165 (25.1%) were primary school leavers, 231 (35.2%) ended their education at the secondary level, and 30 (4.5%) had tertiary education (Table 1).

Prevalence of *Staphylococcus aureus* among study participants

From Table 2, the number of isolates confirmed to be *S. aureus* was 294 of 657 samples, representing 44.7%. The most affected age group was found among 30–39 in which 52.6% of the group were infected with *S. aureus*, whereas none in the 10–19 age group was infected. Association was found between *S. aureus* and age in years ($p = 0.001$). However, no significant association between *S. aureus* and educational status ($p = 0.239$), religion ($p = 0.504$), sex ($p = 0.77$), and marital status (0.67) was found. There was no significant difference in colonization between outpatients and hospitalized patients ($p = 0.266$) (Table 2).

Prevalence of MRSA among HIV patients

From Table 3, the MRSA prevalence was found to be 8.2% (54/657) and the MSSA was 91.8% (603/657). Participants who were hospitalized showed a significant increase in MRSA colonization ($p = 0.04$) compared to nonhospitalized patients. Moreover, an association was found between sex ($p = 0.031$) and

TABLE 1 Sociodemographic distribution of study participants at Cape Coast Teaching Hospital from January to May 2018.

Characteristics	Category	N	%
Sex	Men	204	31.1
	Women	453	68.9
Age groups	10–19	18	2.7
	20–29	84	12.8
	30–39	171	26
	40–49	228	34.7
	50–59	126	19.2
	60–69	24	3.7
	70–79	6	0.9
Educational level	Primary	165	25.1
	Secondary	231	35.2
	Tertiary	30	4.5
	Uneducated	231	35.2
Religion	Christianity	447	68
	Islamic	210	32
Hospitalization	Yes	351	53.4
	No	306	46.6
Marital Status	Divorced	141	21.5
	Married	171	26
	Single	345	52.5

MRSA, where more men (11.8%) carried MRSA as against women (6.6%). There was no significant association found between the educational level (0.514) of the participants, their religion (0.545), marital status (0.111), and age (0.85) (Table 3).

Antibiotic susceptibility pattern of *S. aureus* isolates

Antimicrobial susceptibility performed on *S. aureus* isolates against commonly used antimicrobials showed percentage resistance for ampicillin, cefotaxime, cephalexin, ciprofloxacin, cloxacillin, gentamycin, lincomycin, ofloxacin, prulifloxacin, tetracycline, and vancomycin as 69.4%, 33.7%, 30.6%, 39.8%, 39.8%, 27.6%, 37.7%, 55.1%, 42.9%, 74.5%, and 14.9%, respectively (Figure 1). The most commonly used antimicrobial, i.e., co-trimoxazole, had the most resistance of 94.9%, whereas mupirocin was the most effective (100%) (Figure 1).

Discussion

MRSA colonization and infections have been increasingly reported in HIV patients. The reason for this high prevalence or rate of colonization is not clear, but it has been associated with frequent contact with healthcare workers (11, 12), geographical locations, intermittent MRSA colonization, and prevalence of ART in the study population (13).

The results of this study indicate the *S. aureus* prevalence among HIV patients (44.7%) in Cape Coast, which was similar (44.9%) to a study done by (14) in Ghana. However, their study was conducted in a different geographical study (Accra) and on a different target population, i.e., HIV-infected children under the

TABLE 2 Distribution of *Staphylococcus aureus* in study participants at Cape Coast Teaching Hospital from January to May 2018.

Variables	Frequency (%)	*S. aureus* colonization			
		Yes (%)	No (%)	OR (95% CI)	P-value
Age in years					
10–19	18 (2.7)	0 (0)	18 (100)	NC	0.001
20–29	84 (12.8)	33 (39.3)	51 (60.7)		
30–39	171 (26)	90 (52.6)	81 (47.4)		
40–49	228 (34.7)	96 (42.1)	132 (57.9)		
50–59	126 (19.2)	63 (50)	63 (50)		
60–69	24 (3.7)	9 (37.5)	15 (62.5)		
70–79	6 (0.9)	3 (50)	3 (50)		
Sex					
Women	453 (68.9)	201 (44.4)	252 (55.6)	1.05 (0.75–1.46)	0.77
Men	204 (31.1)	93 (45.6)	111 (54.4)		
Education Level					
Primary	165 (25.1)	75 (45.5)	90 (54.5)	NC	0.239
Secondary	231 (35.2)	94 (40.7)	141 (59.3)		
Tertiary	30 (4.6)	11 (36.7)	15 (63.3)		
Uneducated	231 (35.2)	114 (49.4)	117 (50.6)		
Religion					
Christianity	447 (68)	204 (45.6)	243 (54.4)	1.12 (0.81–1.56)	0.504
Islamic	210 (32)	90 (42.9)	120 (57.1		
Hospitalization					
Yes	351 (53.4)	150 (42.7)	201 (57.3)	0.84 (0.62–1.14)	0.266
No	306 (46.6)	144 (47.1)	162 (52.9)		
Marital Status					
Single	345 (52.5)	153 (44.3)	192 (55.7)	NC	0.67
Married	171 (26)	81 (47.4)	90 (52.6)		
Divorced	141 (21.5)	60 (42.6)	81 (57.4)		
Total	**657**	**294 (44.7)**	**363 (55.3)**		

NC, Not computed; S. aureus, Staphylococcus aureus; OR, odds ratio; Cl, Confidence interval; MRSA, Methicillin resistant Staphylococcus aureus; POS, Positive; NEG, Negative.
Bold values means the total indicates the total number of participants in the second which is 657 (100), followed by the colonisation of S. aureus 294 and then finally followed by participants not colonised with the microbe 363.

TABLE 3 Prevalence of MRSA among HIV patients by sociodemographic characteristics.

Variables	Total	MRSA			
		POS n (%)	NEG n (%)	OR (95% CI)	P-value
Age in years					
10–19	18	0 (0)	18 (100)	NC	0.85
20–29	84	6 (7.1)	78 (92.8)		
30–39	171	18 (10.5)	153 (89.5)		
40–49	228	18 (7.9)	210 (92.1)		
50–59	126	9 (7.1)	117 (92.9)		
60–69	24	3 (12.5)	21 (87.5)		
70–79	6	0 (0)	6 (100)		
Sex					
Women	453	30 (6.6)	423 (93.4)	0.53 (0.29–0.94)	0.031
Men	204	24 (11.8)	180 (88.2)		
Education Level					
Primary	165	11 (6.6)	154 (93.4)	NC	0.514
Secondary	231	17 (7.3)	214 (92.7)		
Tertiary	30	2 (6.6)	28 (93.4)		
Uneducated	231	24 (10.3)	207 (89.7)		
Religion					
Christianity	447	39 (8.7)	408 (91.3)	1.243 (0.68–2.36)	0.545
Islamic	210	15 (7.1)	195 (92.9)		
Hospitalization					
Yes	351	36 (10.3)	315 (89.7)	1.82 (1.01–3.36)	0.04
No	306	18 (5.9)	288 (94.1)		
Marital Status					
Divorced	141	9 (6.4)	132 (93.6)	NC	0.111
Single	171	18 (10.5)	153 (89.5)		
Married	465	27 (5.8)	438 (94.2)		
Total	**657**	**54 (8.2)**	**603 (91.8)**		

NC, Not computed; OR, odds ratio; Cl, Confidence interval; MRSA, Methicillin resistant Staphylococcus aureus; POS, Positive; NEG, Negative.
Bold values means total indicates the total number of participants, followed by the participants colonised with the MRSA which is 54 (8.2) and then followed 603 participants who were colonised with MRSA.

age of 15 years. Our study, on the other hand, targeted a wider range of participants aged between 10 and 80 years, but, interestingly, the participants in the 10–19 age group accounted for 0% prevalence of *S. aureus*. However, the participants in the 30–49 age group had as high as 52% prevalence of *S. aureus*. This shows that participants from this age group may be in danger of being attacked by invasive infection. Another similar study also conducted in Accra by Egyir et al. (15) revealed 8% (10/124) *S. aureus* prevalence in HIV patients with a mean age of 41 years. This result could be associated with the fact that the participants in the study of Egyir et al. were from the Greater Accra Region and received antibiotics, whereas those in this study were from the Central Region and did not receive antibiotics. Generally, HIV patients in the Greater Accra Region are more knowledgeable about HIV and AIDS than those in the Central Region (16). In another study, (17) observed different studies at different times and summarized that nasal *S. aureus* carriage ranged between 36.9% and 45.8%. In our study, the association between age (in years) and *S. aureus* colonization was significant ($p = 0.001$) as individuals between 40 and 49 years carried 52.6%, whereas those between 10 and 19 carried 0% of the bacteria.

MRSA isolates were detected using their resistance (≤21 mm) to cefoxitin according to CLSI guidelines. The prevalence of 8.2% obtained in this study was lower than those that have been reported around the world. For instance, the prevalence of 28.6% was reported in Nepal (18), between 18.3% and 42% in India (19), 16.1% in Nigeria (20), and 21% in South Africa (13). However, the prevalence of MRSA found in this study was high as compared to the studies conducted in Ghana: 0% reported by Egyir et al. (15), 3.4% reported by (14), and 5.6% reported by (7). This high prevalence suggests that geographical location may have an effect on MRSA prevalence because our study was conducted in Cape Coast as compared to those in Accra. From the previous studies, it could also be found that MRSA prevalence has been increasing from 0% in 2016 (15), 3.4% in

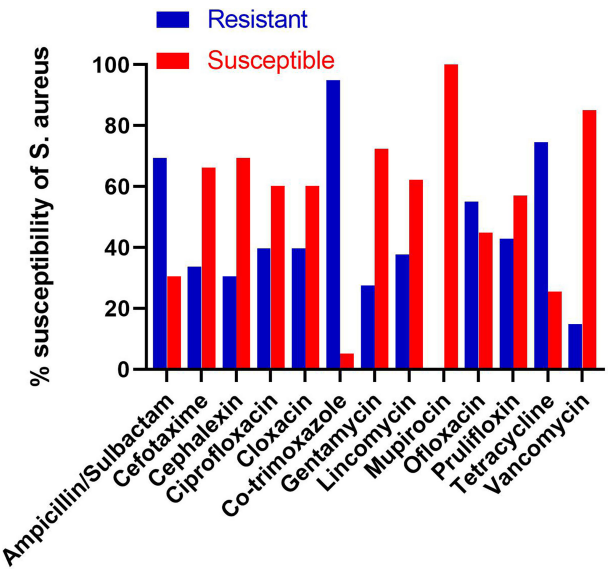

FIGURE 1
Antimicrobial susceptibility and resistance patterns of *Staphylococcus aureus* isolated from nasal swabs of HIV patients in CCTH.

2017 (14), to 5.6% in 2019 (7), which does not make 8.2% in the present study surprising. It is a well-established fact that hospitalization facilitates the colonization of MRSA as indicated in this study (p = 0.04), which may be due to continuous contact with contaminated fomites and other patients or healthcare givers carrying the bacteria. Again, this study did not record vancomycin resistance among the MRSA strains. This is consistent with a study by Kotey (21). The absence of resistance of vancomycin in MRSA therefore suggests that vancomycin remains effective in the treatment of MRSA infections in Ghana.

In this study, men (45.6%) carried more *S. aureus* than women (44.4%) which corroborates the findings reported by Odu and Okonko (22), which also showed a higher percentage of men carrying the bacteria than women even though no association was found (p = 0.77). However, there was a significant difference in the carriage rate of MRSA between the two genders (p = 0.031), where 11.8% of men were colonized with the methicillin-resistant strains as compared with 6.6% in women as was similarly observed in the report of Grundmann et al. (23). Illiteracy is a factor for increased risk of MRSA for persons living with HIV/AIDs (24, 25). These studies observed that literates with a tertiary level of education had low MRSA colonization compared with illiterates and participants with primary education. This result is supported by the report of Aaron and colleagues in which they admonished that individuals with HIV/AIDS should get appropriate knowledge about the unstable nature of their immune system to prevent underlying infections such as MRSA (26). Hospitalization has been considered a risk factor for nasal MRSA colonization in HIV patients (27). This means that healthcare centers can be plausible centers for infection, and, as such, it is prudent for patients to receive proper care during their hospitalization period to prevent morbidity and mortality or being carriers after visiting the hospital. Our results showed a significant difference between hospitalized patients and nonhospitalized patients (p = 0.002), which is consistent with several reports done elsewhere (28). There is no particular study that correlates age and bacterial colonization, but young individuals have been reported to be at a higher risk. In our study, there was a significant difference in MRSA colonization in ages (p = 0.001). Individuals between the ages of 60 and 69 years had the most colonization (12.5%) of the total MRSA colonization in this study. The result is not similar to the study by Hassanzadeh and colleagues who reported a high risk of colonization for a mean age of 40 years.

The antimicrobial resistance of the *S. aureus* including MRSA to selected antimicrobials showed increased resistance to most of the antibiotics especially co-trimoxazole (94.9%) and tetracycline (74.5%). High resistance to co-trimoxazole could be a result of the increased use of the drug because it is not a prescription drug and is mostly given to HIV patients as a prophylactic drug. The excess intake, therefore, might have exerted selected pressure on the microbes leading to resistance. Tetracycline is an overly exploited drug because it is used to prevent bacterial infections in livestock and to promote the growth of the animals (29). Given this, the consumption of uncooked meat could lead to the transfer of resistant genes to other bacteria. The resistance of these two drugs is high

compared with those reported by (7) and Egyir et al. (15). The most effective drugs in this study were found to be mupirocin and vancomycin with sensitivities of 100% and 85.1%, respectively. Mupirocin and vancomycin are two of the few antibiotics that are hardly used in self-medication, hence their high effectiveness. The resistance prevalence in mupirocin corroborates the findings in the study conducted by Egyir et al. (6).

This study has revealed the prevalence of *S. aureus* and MRSA among HIV-infected individuals in Cape Coast to be 44.7% and 8.2%, respectively. Being aged between 30 and 49 and being male are risk factors for both *S. aureus* and MRSA colonization. Also, hospitalization increases one's risk of carrying MRSA. The susceptibility of the isolates to gentamycin, vancomycin, and mupirocin means that the antibiotics should be in the treatment regimen for respiratory infections caused by *S. aureus*.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

This study was reviewed and approved by Research and Developmental Secretariat of CCTH (Ref: CCTH/RDS/2017/03). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

DB and PB were involved in conception and had oversight and leadership responsibility for the research work. DB, SAA, SOA, SK, and PB were involved in the design of study and the questionnaire. SAA, SOA, and SK were involved in the selection of participants and sample collection and carried out experiments. DB, SAA, SOA, SK, and PB participated in data analysis, description of results, and interpretation of data. DB, SAA, SOA, SK, and PB contributed to the original draft or revised it critically for important intellectual content. DB, SAA, SOA, SK, and PB edited and approved this manuscript for submission. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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