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Perspective for Methicillin-resistant Staphylococcus Aureus colonization, Antibiotic Susceptibility Patterns and Risk factors for Colonization among People Living with HIV at Nyenga Hospital, Buikwe District, in Central Uganda

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Abstract

Background: Colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) is recognized as an association towards development of infections that may cause of morbidity among people living with Human Immunodeficiency Virus (PLWHIV). We report on the prevalence, antibiotic susceptibility pattern and risk factors associated with MRSA carriage among PLWHIV at Nyenga hospital, Buikwe district in central Uganda.

Materials and Methods**:** We conducted a cross-sectional study among PLWHIV attending Nyenga hospital anti-retroviral therapy (ART) clinic. Nasopharyngeal swab was collected from each participant, cultured to isolate *Staphylococcus aureus*, and drug susceptibility testing (DST) performed. Sociodemographic data and medical history was recorded.

Results: We enrolled 219 PLWHIV; of these, 58.4% (N=128) were females. The majority of participants (95.0%) were on ART. Ninety-eight (44.75%) of the nasopharyngeal swabs had growth, of which 41 (41.84%) were *S. aureus.* Of these, 11 (5.02%, 95% confidence interval: 3.67-7.02) were MRSA. Of 41 isolated *S. aureus* strains, only 8 (19.51%) were susceptible to all antibiotics tested. A total of three (7.32%) were multi-drug resistant (MDR), while one1 (2.43%) was a possible extensively drug resistant (XDR) strain. Deteriorating immunologic state as indicated by a low CD4 count showed a significant association with the MRSA colonization.

Conclusion: These results are reassuring that MRSA colonization is high among PLWHIV. As most of the antibiotics in use were resistant, it raises concerns of intricate clinical management in a low resource set up.

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Background

Staphylococcus aureus is associated with both nasocomial and community acquired infections [1]. It is categorized as either methicillin-resistant S. *aureus (*MRSA), or methicillin-susceptible, based on the antimicrobials a strain is susceptible to *in vitro* [2]. MRSA poses a public health challenge, in part because of its commensal nature on human skin, and mucous membranes [3, 4]. As such, MRSA is readily passed from person-to-person, especially in hospitals), and is often difficult to eradicate [5-7]. At the same time, Antibiotic pressure has led to regular and unwittingly demands for antibiotic use, which has increased the risk of methicillin resistance [8, 9]. MRSA strains acquire resistance to beta-lactams and aminoglycosides, the common empirical regimens in sub-Saharan Africa [10, 11]. Knowledge of locals antimicrobial susceptibility pattern is essential to select appropriate therapy; however lack of culture and susceptibility testing at most health facilities in resource-limited settings has resulted in widespread empiric therapy. Due to budgetary constraints and lack of programs supporting antimicrobial stewardship, screening for MRSA carriage among PLWHIV in Uganda is not done. This study reports the prevalence, risk factors and DST of asymptomatic MRSA nasal carriage among PLWHIV attending ART clinic at Nyenga hospital in central Uganda.

Methods

Study Design, Area and Population

This cross-sectional study was conducted among PLWHIV attending Nyenga hospital ART clinic between July and November, 2017. Nyenga hospital is a Catholic-founded, private, community not-for-profit facility located in Nyenga town in central Uganda, approximately 15 kilometers west of Jinja. It receives patients from the districts of Buikwe, Jinja, Buvuma and Mukono. The study enrolled consented HIV seropositive clients who were ³18 years of age.

Specimen Collection and Analysis

All study participants had their bilateral anterior nares swabbed with a sterile Dacron dual-swab (*Copan Italia Brescia, Italy*) which was labeled with a unique identifier. Swabs were transported in a cooler box maintained at 2-8^{0}C to the teaching laboratory of Clarke International University (Formerly, International Health Sciences University) for analysis. The dual swab was removed, allowed to equilibrate to room temperature (23-25^{0}C) and inoculated directly onto blood agar and MacConkey agar for 24hrs (*Fisher, Leicestershire, United Kingdom*). After 24 hours, the cultures were checked for growth, and results were recorded as positive or negative. The positive cultures were classified by colony size and morphology, color, and zones of clear beta hemolysis on the media as described [12-14]. A Gram stain was prepared from growth isolates consistent with *Staphylococcus* species. Biochemical testing was then performed, and Gram-positive cocci that were catalase positive were presumptively identified as *Staphylococcus aureus*. To confirm bacterial identity, a 0.5 McFarland suspension of the single colony was prepared and then streaked onto CHROMagar-MRSA (*BD Diagnostics, Inc.*), an MRSA-selective medium. For those samples that grew on CHROMagar-MRSA, additional biochemical identification using coagulase test, DNase test and manitol salt agar was done as described [12, 15, 16]. DST was performed by sub culturing a suspension equivalent to 0.5 McFarland standards on to Mueller Hinton Agar (MHA) [17, 18]. *In vitro* drugs tested were penicillin (10µg), gentamycin (10 µg), erythromycin (15 µg), clindamycin (2 µg), vancomycin (30 µg), and cefoxitin (30 µg). Cefoxitin (30 µg) was used as a surrogate marker for MRSA as described in other studies [19-21]. All testing was performed using a standard operating procedure and positive control MRSA strain *ATCC 33591* and MSSA strain *ATCC 25128*.

Data Analysis

Summary statistics were used to describe the cross-sectional cohort, and Chi squared test was used to measure association between variables. Logistic regression analysis was used to determine risk factors associated with MRSA colonization in this population. For all analyses, *a P*-value of less than 0.05 was considered statistically significant.

Ethical Consideration

Ethical approval was sought from the research and ethics committee of Clarke International University (Formerly called International Health Sciences' University). Written informed consent was obtained from study participants.

Results

We enrolled 219 PLWHIV, and 58.4% (N=128) were female. The majority of participants were aged 26 to 35 years, *(n*=86, 39.27%), and 95.0%) were on ART (Table 1).

There were 98 (44.75%) cultures which exhibited growth. Of those, 41 (41.84%) isolates were of *Staphylococcus aureus.* Of these, 11 participants were found to have MRSA as measured by resistance to Cefoxitin, giving a prevalence of 5.02% (95% confidence interval: 3.67-7.02). Microbiologic results are described in Figure 1.

Analysis of the factors associated factors indicated that low CD4 cell counts and deteriorating World Health Organization (WHO) clinical stages were significantly associated with the risk of MRSA colonization. Participants whose CD4 cell counts were less than 500 cells/litre ($p<0.05$) and WHO clinical stages II ($p=0.000$), III ($p=0.012$) and IV ($p=0.001$) were statistically associated with MRSA colonization, as given in table 2.

DST testing revealed that of 41 isolated *S. aureus* strains, only 8 (19.51%) were sensitive to all the antibiotics tested, including penicillin. A total of 3 (7.32%) were multi-drug resistant, while 1(2.43%) was a possible XDR in regard of the antibiotics used. This study has revealed that all *Staphylococcus aureus* was susceptible to vancomycin 41/41 (100%), than clindamycin 24/41 (58.54%), cefoxitin 20/41 (48.78%), erythromycin 14/41 (34.15%) and penicillin 8/41 (19.51%). The susceptibility pattern for clindamycin indicated that 3/17 (17.65%) were inducible resistances, while 14/17 (82.35%) were constitutively resistant. The resistance to erythromycin was 2/46 (4.35%) among all *Staphylococcus aureus* isolates. The MRSA isolates were more sensitive to vancomycin with 100% sensitivity, while the rest of the antibiotics were poor to gentamycin, clindamycin, erythromycin and penicillin which all gave 100% resistance to MRSA isolates, as presented in Table 3.

Discussion

To the best of our knowledge, and search, this is the first report of MRSA colonization among PLWHIV attending Nyenga Hospital in Buikwe district. The prevalence of MRSA reported here was 5.02% (95% confidence interval: 3.67-7.02), comparable to the 5.1% reported in Singapore [22], and slightly higher than 2% that was reported in Spain [23] and 2.4% in northern Ethiopia [24]. On the other hand, the prevalence is lower than 15.4% reported among an adult cohort from Johns Hopkins University AIDS Service in Baltimore [25], and 16.8% in Northeast Ethiopia [26]. The low prevalence of MRSA colonization in this study is ascribed to the occasional visit of PLWHIV to the health facility, since repeated visits or contact with hands of health workers in HIV infected individuals is the major risk factor for colonization [24, 25].

Low CD4 cell counts [less than 200 ($p=0.000$) and less than 500 cells/litre ($p=0.003$)] and deteriorating WHO clinical stages were significantly associated with MRSA colonization. This finding affirms to results from previous studies [5, 25]. Although mechanisms of resistance were unable to be further characterized, it is notable that HIV-infected clients with advanced immune suppression were more likely to carry MRSA. This is consistent with what was reported from other studies [5, 26, 27].

The assessment of the drug susceptibility pattern of the MRSA isolates indicated high rates of co-resistance of MRSA to commonly prescribed antibiotics such as gentamycin, clindamycin, erythromycin and penicillin which all gave 100% resistance. This is similar to earlier reports [4, 28]. The observed high rates of resistance to penicillin, gentamycin, erythromycin, clindamycin, and vancomycin suggest that most of this colonization were caused by resistant strains [28, 29]. The high rates of concomitant drug resistance to the commonly available reserve antibiotics for use among the HIV population is of critical attention as *S. aureus* is a common pathogen among PLWHIV [26]. As all participants were ambulatory, those colonized by MRSA could easily transmit the pathogenic bacteria to the community. Our study findings subject to the following limitations, including that nares are not the only primary body site of colonization, so our reported prevalence could under-detect MRSA colonization in this population. In addition, we were unable to genetically characterize MRSA isolates due to resource constraints.

Conclusions:

Our results are reassuring that the overall MRSA colonization is high in our setting. As the majority of the

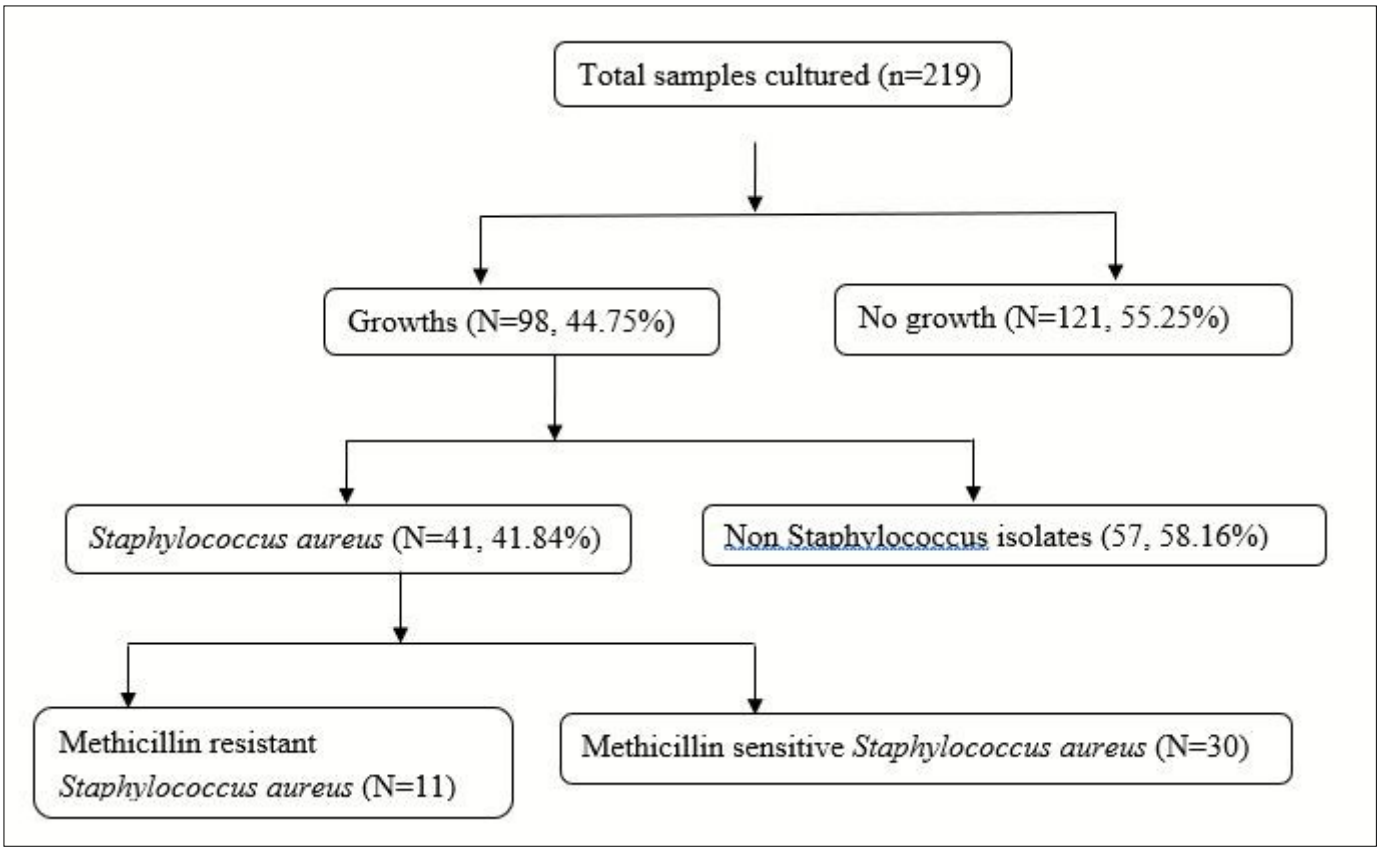

Table 3. Antibiotic susceptibility patterns of S. aureus isolates tested to each antibiotic

Drug agent	Strain	Resistant strains	Sensitive strains	Odds ratio	p-value
Vancomycin (30µg)	MRSA	0	11(26.83)		
	MSSA	0	30 (73.17)		
Clindamycin (2µg)	MRSA	11 (26.83%)	0	1.32	0.162
	MSSA	2 (4.88%)	28 (68.29%)		
Erythromycin (15µg)	MRSA	11 (26.83%)	0	1.98	0.388
	MSSA	16 (39.02%)	14 (34.15%)		
Gentamycin (10µg)	MRSA	11 (26.83%)	0	1.69	0.511
	MSSA	2 (4.88%)	28 (68.29)		
Penicillin (10µg)	MRSA	11 (26.83%)	0	0.27	0.102
	MSSA	22 (53.66%)	8 (19.51%)		

Table 1. Socio-demographic characteristics of study participants

Variable	Frequency	Percentage
Age group (Years)	63	28.77
18-25	86	39.27
26-35	33	15.07
36-49	37	16.89
Above 49		
Education level		
None	51	23.29
Primary	83	37.9
Secondary	69	31.51
Tertiary	16	7.31
CD_4^+ cell counts (/Litre)		
0 – 199	84	38.36
200 - 499	132	60.27
Above 500	3	1.37
Taking ART		
Yes	208	95.0
No	11	5.0
WHO Clinical stage		
I	41	18.72
II	78	35.62
III	55	25.11
IV	45	20.55

Table 2. Socio-demographic and medical factors associated with MRSA colonization

MRSA colonization			
Variable	Absent, n= (%)	Present, n= (%)	P-value (95% CI)
Age group (Years)			
18-25	62 (29.81)	1 (9.09)	1
26-35	82 (39.42)	4 (36.36)	0.531 (1.011-1.306)
36-49	27 (12.98)	6 (54.55)	0.600 (0.576-1.621)
Above 49	37 (17.79)	0 (0.0)	0.150 (0.162-0.197)
Education level			
None	45 (21.63)	6 (12.9)	1
Primary	79 (37.98)	4 (36.36)	0.300 (0.248-1.454)
Secondary	79 (37.98)	4 (36.36)	0.080 (0.373-1.804)
Tertiary	15 (7.21)	1(9.09)	0.210 (0.159-2.062)
CD_4^+ counts (/Litre)			
0 - 199	76(36.54)	8(72.73)	0.000*(0.059-0.676)
200 - 499	129(62.02)	3.(27.27)	0.003* (0.055-0.456)
Above 500	3(1.44)	0(0.00)	1
WHO clinical stage			
I	40 (19.23)	1 (9.09)	1
II	75 (36.06)	3(27.27)	0.000* (0.018-0.171)
III	53 (25.48)	2 (18.18)	0.012* (0.023-0.122)
IV	40 (19.23)	5 (45.45)	0.001* (0.000-0.164)
CI denotes Confidence Interval; 1 is the reference group			

antibiotics in use were resistant, it raises significant concerns of very complicated clinical management. Deteriorating immunological state as measured by low CD4 cell count and advanced clinical stages of infection were significantly associated with MRSA colonization. Premised on these, we have demonstrated the need to encourage routine MRSA screening because of the associated factors.

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Conflict of Interests

The authors declare no conflict of interest in this work.

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