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Original article

Characterization of *Staphylococcus aureus* from Human Immunodeficiency Virus (HIV) patients in Accra, Ghana

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Introduction: The aims of this study were to: a) determine the nasal carriage prevalence of *Stahpylococcus aureus* among HIV patients, b) to characterize *S. aureus* strains isolated.
Methodology: Characterization of *S. aureus* isolates was done by antibiotyping, *spa* typing, and detection of Panton-Valentine leukocidin (PVL) genes.
Results: *S. aureus* isolated (10/124; 8%) belonged to *spa* types t084 (n = 3), t10828 (n = 2), t311, t304, t774, t645, and t091. The isolates were resistant to penicillin (100%), tetracycline (40%), rifampicin (10%), fucidic acid (10%), norfloxacin (10%), erythromycin (10%), and sulfamethoxazole trimethoprim (10%). Multidrug resistance (MDR) was detected in 30% of the isolates.
Conclusion: The finding of MDR *S. aureus* among HIV-positive patients suggests that surveillance of antimicrobial resistant *S. aureus* among this patient group could be considered as an infection control measure in the hospital.

Key words: *Staphylococcus aureus,* nasal carriage, HIV patients, Africa

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Introduction

Among human immunodeficiency virus (HIV) infected patients, nasal carriage of *Staphylococcus aureus* is a recognized risk factor for infections with significant morbidity and mortality [1,2]. Characterization of colonizing *S. aureus* strains in this group of patients is therefore of clinical importance. Data on antimicrobial resistance in *S. aureus* among HIV positive patients from Africa, the continent with the highest burden of HIV infection; often co-infected with tuberculosis [3] have mainly originated from Nigeria [2], South Africa [4,5] and Kenya [6]. The study from Nigeria indicated carriage prevalence of 33% and 5.6% of *S. aureus* and MRSA among HIV-infected patients [2]. These isolates were commonly resistant to potentiated sulfonamides; *spa* types t064 and t3772 were frequently detected among isolates with high (32%) occurrence of Panton-Valentine leukocidin (PVL) from this patient group in this region. PVL is a toxin associated with necrotizing pneumonia and skin and soft tissue infection [7]. A recent study showed *S. aureus* and MRSA carriage prevalence of 14% and 10% among inpatients (not diagnosed with HIV) at Korle Bu teaching hospital in Ghana [8]. In this same geographic setting, *spa* type t355 and t084 together with high prevalence of PVL (21-60%) were predominantly detected [8,9]. There is however, no information on *S. aureus* carriage strains among HIV-infected patients in Ghana. The objective of this study was therefore to: i) determine the nasal carriage prevalence of *S. aureus* among HIV patients at the largest teaching hospital in Ghana, ii) to determine the antimicrobial resistance and clonal diversity of the colonizing *S. aureus* isolated.

Methodology

Study design, area and participants

This cross sectional study was conducted between July and August 2011 among clinically diagnosed HIV out and inpatients receiving care at the fevers unit of the Korle Bu teaching hospital (KBTH). This hospital is the largest in Ghana and serves an estimated population of 24 million people. The fevers unit has 27 beds, and an average outpatient attendance of 73 on clinic days (Mondays, Wednesdays and Fridays). Nasal samples were obtained after receiving an informed consent from study participants; descriptive data such as age, sex,

status (HIV/ HIV/TB co-infections), and antimicrobial therapy were obtained.

Isolation of S. aureus and antimicrobial sensitivity testing

Nasal swabs were processed as described previously [8]. *S. aureus* isolates were identified as described elsewhere [8]. Antimicrobial susceptibility testing was performed and interpreted according to EUCAST guidelines (www.eucast.org) using (gentamicin (10µg), rifampicin (5µg), linezolid (10µg), fusidic acid (10µg), cefoxitin (30µg), norfloxacin (10µg), tetracycline (30µg), clindamycin (2 µg), erythromycin (15 µg), penicillin (1unit), and sulfamethoxazole trimethroprim (1.25+23.75 µg). *S. aureus* strain ATCC 29213 was included as control. Isolates resistant to at least three distinct antimicrobial classes were classified as MDR [10].

Molecular characterization

Detection of the *spa,* Panton-Valentine leukocidin (*pvl*) and *mecA* genes was done in a multiplex polymerase chain reaction [11]. The Ridom *spa* server (http://*spa*.server.ridom.de) plug-in together with BioNumerics v.6.5 (Applied Maths, Sint-Martens-Latem, Belgium) was used to assign *spa* types and multi locus sequence clonal complexes.

Statistical analysis

Data were analyzed using Microsoft excel and R software (version 0.98.93, RStudio Inc). Prevalence was expressed as percentages. Logistic regression analysis was used to determine the association between *S. aureus* carriage and other variables investigated. P values <0.05 were considered significant in a 95% confidence interval.

Results and Discussion

In this study, a total of 124 HIV infected patients (mean age: 41) were screened. Of these, 68% (n = 84) were females, 86% (n = 107) were outpatients and 61% (n = 76) were co-infected with tuberculosis. Table 1 shows the demographic characteristics of study participants. *S. aureus* was isolated from ten (10/124; 8%) patients; six of these isolates were detected in patients with HIV/TB co-infection. By contrast, higher *S. aureus* nasal carriage prevalence (25-33%) have been reported among HIV patients in other African studies [2,4]. The low number of positive patients did not allow analysis for relation between carriage and demographic characteristics of the patients.

Table 1. Demographic characteristics of *S. aureus* carriers among HIV patients at Korle Bu Teaching Hospitals, Ghana, 2011.

		***S. aureus* carriage**			
Characteristics	Category	Carriers n (%)	Non carriers n (%)	Total n (%)	*p*-value
Age (mean±SD)		45.3±8.5	40.6±10.7	41.0±10.6	0.1793
Sex	F	6 (60.0)	78 (68.4)	84 (67.7)	
	M	4 (40.0)	36 (31.6)	40 (32.3)	0.8466
Diagnosis	HIV	4 (40.0)	72 (63.2)	76 (61.3)	
	HIV/ TB	6 (60.0)	42 (36.8)	48 (38.7)	0.2700
Antimicrobial Therapy	No	4(40)	19 (16.7)	23 (18.5)	0.1627
	Yes	6(60)	95 (83.3)	101 (81.5)	
Ward	OPD	10 (100.0)	97 (85.1)	107(86.3)	
	IP	0 (0.0)	17 (14.9)	17 (13.7)	0.4036

Abbreviations: sd, standard deviation; HIV, human immunodeficiency virus-infected patients; TB, tuberculosis patients; OPD, outpatient department; IP, inpatient.

Table 2. Characteristics of *S. aureus* isolated from HIV-patients at Korle Bu Teaching Hospital, Ghana, 2011

ID	Age (years)	Sex	Status	Antibiotype	CC	*spa*	PVL
A	43	F	HIV/TB	pen	CC5	t311	-
B	42	M	HIV	pen-ery	CC8	t304	-
C	56	F	HIV/TB	pen	CC15	t084	-
D	55	M	HIV/TB	pen	CC15	t084	-
E	50	M	HIV/TB	pen	CC15	t084	+
F	55	F	HIV/TB	pen-tet-stx	CC15	t774	-
G	33	F	HIV	pen-tet	CC121	t645	-
H	46	M	HIV	pen-tet-nor	CC121	t091	-
I	38	F	HIV	pen-tet-rif-fuc	CC152	t10828	+
J	35	F	HIV/TB	pen	CC152	t10828	+

Abbreviations: PVL: Panton-Valentine leukocidin, CC: clonal complex, HIV: Human immunodeficiency virus-infected patient. TB: Tuberculosis patient, pen: penicillin, ery: erythromycin, tet: tetracycline, nor: norfloxacin, rif: rifampicin, fuc: fucidin, stx: sulfamethoxazole and trimethoprim.

The low number of *S. aureus* positive patients may be due to the fact that 71.4% of HIV patients received cotrimoxazole (septrin) as a form of prophylaxis against opportunistic infections. All *S. aureus* isolated were susceptible to gentamicin, linezolid, cefoxitin and clindamycin, but resistant to penicillin (100%), tetracycline (40%), rifampicin (10%), fusidic acid (10%), norfloxacin (10%), erythromycin (10%), and sulfamethoxazole trimethoprim (10%). Similarly, high resistance of *S. aureus* nasal isolates to penicillin and tetracyline was reported in a previous study among patients in Ghana [8] and Nigeria [2]. We found less resistance of *S. aureus* to potentiated sulfonamides as compared to the high non-susceptibility of *S. aureus* to sulfamethoxazole trimethoprim recently reported from Nigeria [2] although 71.4% of study participant were documented to have received this antibiotic. This could be as a result of the few numbers of *S. aureus* analysed in this study. No MRSA was detected, however, 30% of the isolates in this study were MDR differing from the observation of 13% MDR prevalence among inpatients and hospital staff in a recent study in this same geographic location [8]. By contrast, high (5.3-21%) MRSA prevalence was reported among TB/HIV patients in South Africa and Nigeria [2,4].

Several factors, such as variations in infection control policies [12] could account for the differences between the findings of this study and other African reports. For example, strict adherence to infection control policies (frequent hand washing with soap and disinfection of hands) was observed at the fevers unit during the study. Other factors could be the small sample size, differences in antimicrobial consumption, and methods used in the different studies in various countries [12].

The ten *S. aureus* isolates were genetically diverse with t084 (n = 3) and t10828 (n = 2) as frequent *spa* types contrary to the predominant *spa* types (t064 and t3772) reported among HIV-infected patients in Nigeria [2]. The finding of *spa* type t084 in multiple patients was anticipated as this lineage was common in earlier hospital and community studies in Ghana [8,9,13]. Surprisingly, *spa* type t355, the frequent clone detected among clinical and non clinical isolates in recent studies in Ghana [8,9,13] was not found among this group of patients suggesting differences in *S. aureus* clone distribution with patient groups in this country. Table 2 shows the molecular characteristics of all *S. aureus* isolates in the study. PVL-postive isolates (3/10, 30%) belonged to CC15 (n = 1) and CC152 (n=2), similar to the observation in earlier studies in Ghana [8,9,13].

The study was limited due to the rather small samples analyzed. Despite this shortcoming, the results of this study offer baseline information for future studies.

Conclusion

To conclude, the detection of MDR *S. aureus* among HIV infected patients calls for regular surveillance and screening for infection control purposes. The findings in this study suggest that this group of patients could be targeted as a means to contain the spread of MDR *S. aureus* in the hospital.

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