Molecular Epidemiology and Antimicrobial Susceptibility of Clinical \textit{Staphylococcus aureus} from Healthcare Institutions in Ghana

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Abstract

The objective of this study was to determine the antimicrobial susceptibility patterns and clonal diversity of clinical \textit{Staphylococcus aureus} isolates from Ghana. A total of 308 \textit{S. aureus} isolates from six healthcare institutions located across Northern, Central and Southern Ghana were characterized by antibiotyping, \textit{spa} typing and PCR detection of Panton Valentine leukocin (PVL) genes. Methicillin-resistant \textit{S. aureus} (MRSA) were confirmed by PCR detection of \textit{mecA} gene and further characterized by \textit{SCCmec} and multi-locus sequence typing (MLST). The prevalence of antimicrobial resistance was below 5% for all agents tested except for penicillin (97%), tetracycline (42%) and erythromycin (6%). Ninety-one \textit{spa} types were found, with \textit{t355} (ST152, 19%), \textit{t084} (ST15, 12%) and \textit{t314} (ST121, 6%) being the most frequent types. Based on established associations between \textit{spa} and MLST types, isolates were assigned to 16 clonal complexes (CCs): CC152 (n = 78), CC15 (n = 57), CC121 (n = 39), CC8 (n = 36), CC5 (n = 33), CC1 (n = 29), CC45 (n = 9), CC88 (n = 8), CC30 (n = 4), CC9 (n = 3), CC25 (n = 2), CC97 (n = 2) CC20 (n = 2), CC707 (n = 2), CC7 (n = 3) and CC522 (n = 1). Most isolates (60%) were PVL-positive, especially those belonging to ST152, ST121, ST5, ST15, ST1, ST8, and ST88. Nine (3%) isolates were MRSA belonging to seven distinct clones: ST88-IV (n = 2), ST250-I (n = 2), ST8-IV (n = 1), ST72-V (n = 1), ST789-IV (n = 1), ST2021-V (n = 1), and ST239-III (n = 1). The study confirmed a high frequency of PVL-positive \textit{S. aureus} in Africa, low prevalence of antimicrobial resistance and high diversity of MRSA lineages in Ghana compared to developed countries and other African countries. The detection of known pandemic MRSA clones in the absence of routine MRSA identification in most Ghanaian clinical microbiology laboratories calls for capacity building to strengthen surveillance and prevent spread of these clones.

Introduction

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is a major concern in clinical medicine due to the importance of \textit{B-lactams} in the therapy of staphylococcal infections and the additional morbidity and mortality for MRSA patients compared to patients infected with methicillin-susceptible \textit{S. aureus} (MSSA) \cite{1}. Despite the importance of MRSA, MSSA are among the most common causative agents of bacteraemia and skin and soft tissue infections (SSTI) \cite{2}. Epidemiological surveillance of MRSA and MSSA is of importance for the development and implementation of infection control programmes. Data on \textit{S. aureus} epidemiology in African countries are limited and a common trait for MSSA strains from various African countries seems to be the carriage of the PVL genes: \textit{bikSF-F-vo} at much higher frequencies (>55%) than in the rest of the world (<10%) \cite{2-6}. The high frequency of PVL among human MSSA strains is of special interest since the most successful community associated (CA) MRSA clones share this genetic marker, and could have MSSA ancestors associated with Africa as recently suggested \cite{5,6}. PVL is associated with SSTI and severe necrotising pneumonia and has been shown to be a characteristic feature of community acquired (CA) -MRSA clones disseminated in Europe and Middle East (ST-80), Australia and South America (ST30-IV), and United States (ST8-IV, also known as USA300) \cite{2,7,8}.

The objective of this study was to investigate the antimicrobial susceptibility and clonal diversity of clinical \textit{S. aureus} isolates from Ghana. Antimicrobial resistance in \textit{S. aureus} has previously been reported from Ghana with findings of a low MRSA prevalence in nasal swabs from patients and health care workers at the Korle-bu Hospital, Accra \cite{9} however, treatment in Ghana is mainly empirical due to a relative lack of appropriate laboratory facilities \cite{10} and therefore only few susceptibility data exists and so far no study has investigated the clonal structure of \textit{S. aureus} in clinical samples. The study was part of a cooperation program on Antibiotic Drug use, Monitoring and Evaluation of Resistance (ADMER) in Ghana under the Danish Ministry of Foreign Affairs.
This program was conceived to strengthen clinical microbiology and surveillance of antibiotic resistance, and ultimately to improve awareness of antimicrobial use in Ghana.

**Materials and Methods**

**Ethics Statement**

Ethical clearance was obtained from the University of Ghana Medical School Ethical and Protocol Review Board (reference no. MS-EI/M.9 - P.3.212010-11).

**Bacterial Isolates**

Staphylococcal isolates from clinical specimens were obtained in a prospective cross-sectional-like study between October 2010–June 2012 from six healthcare institution situated at Northern (Tamale Teaching Hospital), Central (Sunyani Government Hospital) and Southern Ghana (Korle bu Teaching Hospital, Thirty-seven Military Hospital, Ridge Hospital and Legon Hospital) (Figure 1). The majority of the isolates (70%) were obtained from Korle bu Teaching Hospital, which serves a population of over 3 million and acts as a major referral health facility for an estimated population of 24 million people across Ghana. Presumptive staphylococci identified by colony morphology at the hospital clinical microbiology laboratories were collected and sent to Noguchi Memorial Institute for Medical Research, where they were identified as *S. aureus* by Gram staining, catalase, tube coagulase and slidex staphplus test (bioMérieux, Marcy l’Etoile, France). Available patient demographic characteristics such as age and sex were retrieved from laboratory records.

**Antimicrobial Susceptibility Testing**

Susceptibility testing was carried out by disc diffusion technique following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (www.eucast.org) using 1U penicillin, 30 μg tetracycline, 30 μg cefoxitin, 2 μg clindamycin, 15 μg erythromycin, 10 μg norfloxacin, 10 μg gentamicin, 10 μg linezolid, 5 μg rifampicin, 1. 25 μg +23.75 μg trimethoprim-sulfamethoxazole, and 10 μg fusidic acid (Rosco NeoSenstabs, Taastrup, Denmark). Inducible clindamycin resistance was detected by placing clindamycin and erythromycin 12-20 mm apart (D-test). Brain Heart Infusion agar supplemented with teicoplanin (5 mg/L) (Becton Dickinson, Denmark) was used to screen MRSA isolates for glycopeptides resistance by a spot test; if 10 or more colonies were detected on these plates, E-tests (bioMérieux, Marcy l’Etoile, France) were used to determine the minimum inhibitory concentration of vancomycin and teicoplanin [11]. Multidrug resistance (MDR) was defined as resistance to at least three distinct antimicrobial classes or being MRSA [12].

**Molecular Typing**

Molecular characterization of the isolates was done at Statens Serum Institut (SSI), Denmark. A multiplex PCR was used for detection of *spa*, *lukS/F-pv* and *mecA* [13]. *spa* typing was performed as described by Harmsen et al. [14]. Using BioNumerics v.6.5 (Applied Maths, Sint-Martens-Latem, Belgium) with the Ridom *spa* server (http://spa.server.ridom.de) plug-in, *spa* sequences were automatically assigned to *spa* types and clonal complexes (CCs) based on *spa* repeats. Multi-Locus Sequence Typing (MLST) [15] was done on all MRSA and MSSA isolates whose CC could not be assigned by the Ridom *spa* server. Minimum Spanning Tree (MST) based on *spa*-types was made using BioNumerics V6.5 (Applied Maths, Sint-Martens-Latem Belgium). Staphylococcal cassette chromosome mec (SCCmec) typing was performed by multiplex PCR as described previously [16].

**Statistical Analysis**

Distributions of the various genotypes determined in the study (PVL-positivity, *spa* type, ST and CC) were associated to region, hospital, sex and infection type to determine if specific patterns existed. Only genotypes with more than 10 observations were included in statistical analysis. MRSA isolates were not evaluated...
due to the low prevalence of their genotypes. Associations were determined using the \( \chi^2 \) test, except for PVL-positivity, which was analysed by logistic regression. A significant association was deemed at p-values <0.05.

**Results**

Of the 903 presumed staphylococci collected from the six hospitals, 308 (34%) were identified as *S. aureus* and 595 (66%) as coagulase negative staphylococci. *S. aureus* isolates originated from SSTIs (n = 175), bacteraemia (n = 112), and other (urinary tract infection, n = 9; unknown infections, n = 14) infections (n = 23). *S. aureus* was isolated from 143 females and 109 males. Sex origin of 56 isolates could not be traced from laboratory records. With regard to hospital origin, 12 isolates were from Tamale Teaching Hospital (TTH, Northern Ghana), 53 from Sunyani Government Hospital (SGH, Central Ghana) and 243 from the four hospitals in Southern Ghana. Details of hospital location (stratified into regions of study) and proportions of isolates from clinical infections are shown in Figure 1. None of the clinical laboratories used methods for MRSA detection and typing, and several pitfalls were recognized in routine microbiological procedures (e.g. poor identification to species/genus level, and low compliance with international standards for susceptibility testing).

The highest prevalence of resistance was for penicillin (97%), followed by tetracycline (42%) and erythromycin (6%). Lower percentages of resistance were observed for clindamycin (5%), norfloxacin (4%), trimethoprim-sulphamethoxazole (4%), gentamicin (3%), cefoxitin (3%) and fusidic acid (2%). Inducible clindamycin resistance was detected among seven (2%) isolates. Twenty-nine isolates (9%) were MDR, of which 9 (3%) were confirmed mecA positive MRSA. Details of MDR isolates have been shown in Table 1. MRSA isolates were susceptible to vancomycin and teicoplanin. Most of the MSSA isolates (88%, 264/299) were resistant to penicillin (n = 154), penicillin and tetracycline (n = 99) and penicillin and trimethoprim-sulphamethoxazole (n = 11). All isolates were susceptible to linezolid and rifampicin while three isolates (1%) were susceptible to all antimicrobial drugs tested.

High genetic diversity was observed by spa typing, as indicated by the recovery of 91 spa types among all isolates tested. The most common spa types were t355 (19%), t084 (12%), t314 (6%) and t311 (5%). Fifty-six spa types were singletons and eight new spa types were detected: t10809 (ST88), t10810 (ST88), t10811 (ST1), t10837 (ST1) and t10828 (ST152), t10833 (ST152), t10836 (ST1), t10837 (ST1) and t10838 (ST30). A minimum spanning tree, including spa-typing, as indicated previously found to be distribution of spa-types, STs and CCs with regard to infection type, sex, region and hospital of origin. Some spatial variations were observed in the distribution of spa types (e.g. t355 occurred in 34% and 13% isolates from Sunyani Government Hospital and Korle bu Teaching Hospital respectively) but such differences could not be proven to be significant. Isolates from Ridge Hospital (n = 1), Thirty-seven Military Hospital (n = 6) and Tamale Teaching Hospital (n = 12) were excluded from the statistical analysis due to low numbers.

PVL was detected in 60% (n = 184) of the isolates, mainly isolates from SSTIs (57%) and belonging to ST152 (38.5%), ST121 (21%), ST5 (13.5%), ST15 (11%), ST1 (7%), ST9 (3.5%) and ST8 (2.7%). Genotypes and clinical origins of PVL positive *S. aureus* are shown in Table 2. The patterns of PVL varied by region, with Central regions having a higher chance 2.2 (95% CI: 1.1-4.2) of seeing PVL-positive *S. aureus* (p = 0.02) than the Southern region.

**Discussion**

This study fills an important gap in the knowledge of the epidemiology of *S. aureus* in Ghana. As such, the study contributes to the current knowledge of the diversity and population structure of this important bacterial pathogen at the global level. Ghana and several other African countries have so far been black spots on the map due to lack of established national surveillance programmes and adequate clinical microbiology infrastructure [10,17]. Our results show that the most common spa types among MSSA isolates are t355 (ST152) and t084 (ST15). The spa types were previously found to be predominant among *S. aureus* isolates from asymptomatic nasal carriers at Korle bu, the largest Teaching Hospital in Ghana [9], suggesting that they are well established in the human population of this country. In another African study, t084 (ST15) was also reported as one of the most frequent spa types among *S. aureus* isolated from seven tertiary hospitals located in five major African towns [3]. PVL-positive ST152 (t355) is also widely distributed in African countries [4,5] and its frequent recovery from SSTIs is consistent with studies in other countries [4,18]. Other PVL-positive MSSA lineages found in this study such as ST121, ST30, ST15 and ST5 have also been reported elsewhere in Africa [19]. The observed high prevalence (60%) of PVL appears to be a distinguishing genetic trait of African MSSA [3,4,6] compared to USA, Asia and Europe, where this virulence factor is uncommon in MSSA [2,19,20]. This finding was correlated to the high frequency of PVL-positive ST152, which is a likely ancestor of the CA-MRSA ST152-V clone circulating in certain European regions, especially the Balkan area [21,22].

The nine MRSA isolates belonged to seven unrelated spa types and STs harbouring four different SCC*meC* types (Table 1), indicating high clonal diversity. Some of the MRSA lineages identified in this study are widely distributed worldwide: ST239-III is a pandemic clone prevalent in Europe, Asia and South Africa [23–25] and ST789-IV is a single locus variant of the ST7 clone frequently reported in Asia [25]. ST88-IV, ST18-IV and ST72-IV have been previously reported among inpatients and staff at Korle-bu Hospital in Ghana [9] and in communities and hospitals in other African countries [26,27]. MRSA ST88 has been reported sporadically in some European countries like Portugal [28] and Sweden [29]. ST18-IV MRSA (spa type t121, PVL+) found in this study is related to the epidemic MRSA ST8-IV (USA300) clone in the USA [2]. Other African studies have reported this ST8-IV
MRSA (spa type t121, PVL+) strain in communities and hospitals [26,30]. ST250-I, also referred to as the “Archaic clone”, differs from ST8 by a point mutation in the yqiL gene and is related to ST247-I (Iberian clone), a major clone isolated in European hospitals [7,31]. ST72 has been reported as a major MRSA clone from communities in Australia [32] and as MSSA in Nigeria and Gabon [6,27]. The least known MRSA lineage found in this study was ST2021-V, which to the best of our knowledge has previously been reported in a single isolate from Nigeria (www.mlst.net; accessed on: 4th April 2013). Although ST5, ST30 and ST80 MRSA have been described in several African and other countries around the world [26,27], none of these clones were detected among clinical MRSA isolates in Ghana. PVL-positive ST5 and ST30 were however detected among MSSA isolates (Table 2), indicating that these two S. aureus lineages are widespread in African countries, even though acquisition of methicillin resistance seems to be confined to some countries.

The prevalence of antimicrobial resistance in clinical S. aureus isolates from Ghana was generally low. Other African studies have reported similar levels of resistance to penicillin (86%–93%) and tetracycline (28%–48%) but higher levels of resistance to sulphonamides (22%–68%) compared to this study [3,4,9]. Comparatively, the prevalence of MRSA (3%) was lower than those reported in other African countries such as Nigeria (20%) [27], Algeria (45%) [33] and in a multicenter study (15%) involving five major African towns [26]. The low MRSA frequency reported in this study could be attributed to the low consumption of antimicrobial agents such as fluoroquinolones and third generation cephalosporins in Ghana, because they are expensive and are usually prescribed for acute infections [10]. Usage of the afore-mentioned antimicrobial agents has been shown to correlate with an increase in MRSA prevalence [34–36]. The observed MRSA prevalence among clinical isolates in Ghana is similar to those reported in European countries with low MRSA

| ID | Hospital | Infection | CC | ST | spa type | SCCmec | PVL | Antibiotype |
|----|----------|-----------|----|----|----------|--------|------|-------------|
| MRSA 5016 | KB | SSTI | CC1 | ST72 | t537 | V | – | Fox, Pen, Tet |
| 744 | KB | Blood | CC8 | ST2021 | t024 | V | – | Fox, Pen, Tet |
| 2244 | KB | Blood | CC8 | ST239 | t037 | III | – | Fox, Pen, Tet, Fuc, Gen, Cli, Ery, |
| 3464 | KB | Blood | CC8 | STB | t121 | IV | + | Fox, Pen, Nor, Cli, Ery |
| 2207 | KB | SSTI | CC8 | ST250 | t928 | I | – | Fox, Pen, Tet, Gen, Nor,Cli, Ery |
| 2224 | KB | SSTI | CC8 | ST250 | t928 | I | – | Fox, Pen, Tet, Gen, Nor, Cli, Ery |
| 44 | SGH | Unknown | CC88 | ST88 | t186 | IV | – | Fox, Pen, Tet |
| AU81 | SGH | SSTI | CC88 | ST88 | t186 | IV | – | Fox, Pen, Tet |
| 11087 | KB | UTI | CC152 | ST789 | t547 | IV | + | Fox, Pen, Tet, Nor |
| M SSA 2639 | KB | Blood | CC1 | ST1 | t7835 | NA | + | Pen, Tet, Cli, Ery |
| AU93 | SGH | SSTI | CC1 | ST1 | t559 | NA | + | Pen, Tet, Fuc |
| A6 | KB | Unknown | CC5 | ST5 | t311 | NA | + | Pen, Tet, Fuc |
| 5095 | KB | SSTI | CC5 | ST5 | t071 | NA | + | Pen, Tet, Gen, TMS, Fuc, Nor, Cli, Ery |
| T2845 | TTH | SSTI | CC8 | ST8 | t451 | NA | + | Pen, Tet, TMS |
| 1455 | KB | SSTI | CC9 | ST9 | t2700 | NA | – | Pen, Gen, Cli, Ery |
| 1050 | KB | Blood | CC15 | ST15 | t084 | NA | + | Pen, Tet, Gen |
| 2320 | KB | Blood | CC45 | ST508 | t635 | NA | – | Pen, Cli, Ery |
| 1548 | KB | SSTI | CC88 | ST88 | t10809 | NA | + | Pen, Tet, Nor, Cli, Ery |
| 5270 | KB | SSTI | CC88 | ST88 | t10810 | NA | + | Pen, Tet, Nor, Cli, Ery |
| NAB | KB | Blood | CC121 | ST121 | t213 | NA | _ | Pen, Tet, Fuc |
| 3209 | KB | Blood | CC121 | ST121 | t091 | NA | _ | Pen, Tet, Nor, Gen, Cli, Ery |
| 2437 | KB | Blood | CC121 | ST121 | t091 | NA | _ | Pen, Tet, Nor |
| 5293 | KB | Blood | CC121 | ST121 | t314 | NA | + | Pen, Tet, Nor |
| 5775 | KB | Blood | CC121 | ST121 | t314 | NA | + | Pen, Tet, Nor |
| 3984 | KB | SSTI | CC152 | ST152 | t1299 | NA | + | Pen, Tet, Cli, Ery |
| 1544 | KB | SSTI | CC152 | ST152 | t355 | NA | + | Pen, Tet, Cli, Ery |
| 4836 | KB | SSTI | CC152 | ST152 | t355 | NA | + | Pen, Tet, Cli, Ery |
| 112243 | MH | SSTI | CC152 | ST152 | t355 | NA | + | Pen, Tet, Ery |

ST: Sequence Type; CC: Clonal Complex; SCC: Staphylococcal Cassette Chromosome; PVL: Panton-Valentine leukocidin.

*KB: Korle bu Teaching Hospital; SGH: Sunyani Government Hospital; TTH: Tamale Teaching Hospital; MH: Military Hospital.

**SSTI**: Skin and Soft Tissue Infection; **UTI**: Urinary Tract Infection; **TMS**: trimethoprim-sulphamethoxazole.

Pen: penicillin; Fox: cefoxitin; Tet: tetracycline; Nor: norfloxacin; Gen: gentamicin; Fuc: Fucidic acid; Cli: clindamycin; Ery: erythromycin.

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Figure 2. Minimum spanning tree of 308 clinical Staphylococcus aureus isolates from healthcare institutions in Ghana. Nodes indicate spa types and their size shows the relative number of isolates for each spa type. Numbers of frequent (three or more) spa types have been shown. Every colour represents a distinct clonal complex.
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Table 2. Clonal complex (CC), multi-locus sequence type (ST), spa type and clinical origin of 184 Staphylococcus aureus harbouring Panton-Valentine leukocidin (PVL) genes isolated in Ghana, 2010–2012. STs and CCs were inferred from spa types.

| CC   | ST   | spa type (N)                                | Bacteraemia N=65 | SSTI N=104 | Other* N=15 | Total N=184 |
|------|------|--------------------------------------------|------------------|-------------|-------------|-------------|
| CC1  | ST1  | t127 (3), t1931 (3), t593 (1), t559 (1), t10836 (1), t114 (1), t922 (1), t934 (1), t7835 (1) | 6 (9.2)          | 7 (6.7)     | 0 (0.0)     | 13 (7.0)    |
| CC5  | ST5  | t071 (9), t311 (9), t002 (5), t105 (1)     | 12 (18.5)        | 11 (10.6)   | 1 (6.7)     | 24 (13.0)   |
| CC8  | ST8  | t1476 (3), t024 (1), t451 (1), t064 (1), t*121(1) | 2 (3.0)          | 5 (4.8)     | 0 (0.0)     | 7 (3.8)     |
| CC15 | ST15 | t084 (19), t5534 (1), t774 (1)             | 7 (10.8)         | 13 (12.5)   | 1 (6.7)     | 21 (11.4)   |
| CC25 | ST25 | t401 (1)                                   | 1 (1.5)          | 0 (0.0)     | 0 (0.0)     | 1 (0.5)     |
| CC30 | ST30 | t10838 (1), t363 (1)                       | 0 (0.0)          | 2 (1.9)     | 0 (0.0)     | 2 (1.1)     |
| CC88 | ST88 | t2393 (2), t3202 (1), t10809 (1), t10810 (1) | 1 (1.5)          | 4 (3.8)     | 0 (0.0)     | 5 (2.7)     |
| CC121| ST121| t314 (15), t2304 (9), t159 (5), t645 (5), t10777(1), t7002(1) | 14 (21.5)        | 21 (20.2)   | 1 (6.7)     | 36 (19.6)   |
| CC152| ST152| t355 (57), t4690 (3), t1096 (2), t1299 (2), Singletons (11)* | 22 (34.0)        | 41 (39.4)   | 12 (80.0)   | 75 (40.8)   |

SSTI: Skin and Soft Tissue Infection;*Other: UTI: Urinary Tract Infection (n = 5: spa types t355 (3), t547(1) and t5534 (1); Unknown (n = 10; spa types: t311 (1), t645 (1), t4690 (1), t355 (7). *Other spa types associated with CC152: t454, t458, t5268, t*547, t1123, t1172, t5047, t7011, t8821, t10828, and t10833 MRSA.
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prevalence, such as the Scandinavian countries and The Netherlands [37].

Some apparent geographical variations in clonal distribution were observed, but the low number of isolates obtained from the Northern region made comparisons between hospitals or regions meaningless. The clinical information on the 308 S. aureus strains included in the study varied in quality due to incompleteness of the patient records collected from the various hospital clinical laboratories involved in the study. Thus, it was not possible to determine possible associations between antimicrobial therapy and resistance patterns.

We conclude that MRSA occurs at low prevalence among S. aureus investigated in this study. MRSA clones circulating in the country are genetically diverse and a number of them belong to known pandemic clones. The overall levels of antimicrobial resistance are generally low compared to other African countries and to most developed countries, most likely because of the low usage of antimicrobial agents in the country. On the other hand, the study also denotes absence of routine MRSA testing and poor performance standards in most clinical microbiology laboratories in Ghana, highlighting the need for infrastructures to support national antimicrobial policies and surveillance capacity.

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Author Contributions

Conceived and designed the experiments: BE. ARL LG KKA MJN. Performed the experiments: BE. Analyzed the data: BE ARL LG SSN MS. Contributed reagents/materials/analysis tools: AK EF. Wrote the paper: BE ARL LG SSN KKA MJN AK MS.

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