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Prevalence of nasal carriage and diversity of *Staphylococcus aureus* among inpatients and hospital staff at Korle Bu Teaching Hospital, Ghana

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ABSTRACT

There is a paucity of data on *Staphylococcus aureus* epidemiology in Africa. Prevalence of nasal carriage and genetic diversity of *S. aureus* were determined among hospital staff (HS) and inpatients (IP) at the largest hospital in Ghana. In total, 632 nasal swabs were obtained from 452 IP and 180 HS in the Child Health Department (CHD) and Surgical Department (SD). *S. aureus* carriage prevalences were 13.9% in IP and 23.3% in HS. The chance of being a carrier was higher in HS ($P = 0.005$) and IP staying ≤7 days in hospital ($P = 0.007$). Resistance to penicillin (93%), tetracycline (28%) and fusidic acid (12%) was more common than for other agents (<5%). A higher chance of multidrug-resistant *S. aureus* carriage was observed among IP compared with HS ($P = 0.01$). High genetic diversity was shown by *spa* typing, with 55 *spa* types found among 105 isolates; the predominant *spa* types were t355 (10%) and t084 (10%). MRSA was detected in six IP with an overall carriage prevalence of ca. 1.3%, but not in HS. All three MRSA isolates from SD belonged to ST88–SCC*mec* IV, and two of them displayed the same *spa* type and antibiograms; three MRSA isolates from CHD belonged to distinct lineages (ST88–SCC*mec* IV, ST8–SCC*mec* V and ST72–SCC*mec* V). Altogether, these data indicate a high diversity of *S. aureus*, low levels of MRSA carriage, and a higher chance of nasal carriage of multidrug-resistant *S. aureus* among IP compared with HS in this hospital.

1. Introduction

Staphylococcus aureus is an important pathogen associated with human infections in hospitals and communities worldwide [1]. Asymptomatic nasal carriage ranges between 15% and 40% of a population and is an established risk factor for *S. aureus* infection as well as for transmission between patients and healthcare workers [2,3].

Meticillin-resistant *S. aureus* (MRSA) infections are a major healthcare and socioeconomic problem in hospitals worldwide as they are more difficult and expensive to treat compared with infections caused by meticillin-susceptible *S. aureus* (MSSA) strains [4]. Efforts to diminish the spread of MRSA in hospitals are therefore pivotal and may include various infection control initiatives such as improved hand hygiene, rapid diagnostics, and eradication of MRSA in asymptomatic carriers especially prior to surgery [1,5].

Data on carriage, antibiotic susceptibility patterns and the molecular epidemiology of *S. aureus* in the African continent are limited. *S. aureus* carriage prevalences of 17–46% have been found among patients and hospital personnel in Somalia and Sudan [6,7]. A prevalence of 29% was reported by a recent study investigating *S. aureus* carriage in a remote indigenous population in Central Gabon [8]. Data available from different African countries indicate a prevalence of MRSA ranging from 4.8% to 20% in clinical isolates [9–11].

The objective of this large-scale surveillance study was to determine the prevalence of nasal carriage, antimicrobial susceptibility patterns and clonal diversity of *S. aureus* and MRSA among inpatients (IP) and hospital staff (HS) at the largest hospital in Ghana.

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2. Materials and methods

2.1. Study design, site and population

A cross-sectional study was conducted between September 2011 and May 2012 at Korle Bu Teaching Hospital, Ghana, which has over 2000 beds and an average admission of 250 patients daily. The hospital 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.

Nasal screening of IP and HS was conducted at the Child Health Department (CHD) (bed size, 168; staff, 180) and Surgical Department (SD) (bed size, 242; staff, 200), which are situated 200 m apart in different buildings. Clinical conditions of IP in the CHD ranged from febrile illnesses to cancer and various surgical conditions; in the SD, clinical conditions of IP were primarily surgery related. Participation was voluntary and nasal screening was done after receiving informed consent from participants. Descriptive information regarding participant's age, sex, diagnosis, department and period of hospitalisation was collected. History of antibiotic use among IP was retrieved from IP records. Data regarding dosage and duration of antimicrobial therapy were not obtained for this study.

2.2. Sample collection and isolation of S. aureus

Samples were taken by rotating a sterile cotton swab five times in both anterior nares. Nasal swabs were pre-enriched in 5 mL of Mueller–Hinton broth (Oxoid Ltd., Basingstoke, UK) supplemented with 6.5% NaCl, incubated at 37 °C for 24 h and plated on 5% sheep blood agar (Oxoid Ltd.). *S. aureus* isolates were identified phenotypically by colony morphology, haemolysis, catalase test and Gram staining and were confirmed by tube coagulase, Slidex Staph Plus (bioMérieux, Marcy-l'Étoile, France) and PCR amplification of the *spa* gene [12].

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2012 guidelines (http://www.eucast.org) using 30 μg of cefoxitin, 30 μg of tetracycline, 1 U of penicillin, 2 μg of clindamycin, 15 μg of erythromycin, 10 μg of gentamicin, 10 μg of linezolid, 5 μg of rifampicin, 10 μg of norfloxacin, 1.25/23.75 μg of trimethoprim/sulfamethoxazole (SXT), 10 μg of fusidic acid and 200 μg of mupirocin (Neo-Sensitabs; Rosco Diagnostica, Taastrup, Denmark). MRSA isolates were screened for glycopeptide resistance by spot test on brain–heart infusion agar (Becton Dickinson, Denmark) containing teicoplanin (5 mg/L). Isolates demonstrating ≥10 CFUs were subjected to Etest using vancomycin and teicoplanin strips (bioMérieux) as described by Fitzgibbon et al. [13]. Multidrug resistance was arbitrarily defined as resistance of MSSA to three or more distinct antimicrobial classes; MRSA found in this study were included in the multidrug-resistant (MDR) category irrespective of their susceptibility profiles.

2.4. Molecular characterisation

Crude bacterial lysates obtained by boiling for 10 min were used as the DNA template for multiplex PCR detection of *spa*, *mecA* and the *lukF-PV* gene encoding Panton–Valentine leukocidin (PVL), with subsequent sequencing of *spa* amplicons [14]. *spa* types were assigned, clustered and displayed as a minimum spanning tree with the *spa* typing plugin for BioNumerics v.6.5 using default settings (Applied Maths, Sint-Martens-Latem, Belgium). Multilocus sequence typing (MLST) was done on all MRSA isolates as described previously [15], and STs were assigned through the MLST database (http://www.mlst.net/). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed using multiplex PCR assays [16].

2.5. Statistical analysis

Demographic characteristics of IP and HS were described and compared using the χ^2 test. The prevalences of *S. aureus* and MRSA were calculated overall and were stratified by demographic characteristics. Since *S. aureus* carriage is influenced by the duration of hospitalisation (DOH), data were categorised at two levels: ≤7 days and more >7 days, referred to as short and long DOH. The age of study participants was also categorised at two levels: ≤14 years (children) and >14 (adults). The patient's underlying diagnosis was categorised as surgical for IP with surgical conditions and non-surgical for IP without surgical conditions. Logistic regression was used to determine the association of the two outcomes, *S. aureus* and MDR carriage (separately), with department, sex, age group, DOH group, patient underlying diagnosis, and IP or HS status. Data were analysed using the glm-function in R v.2.15.2 [17].

3. Results

3.1. Nasal carriage

Demographic characteristics of the study population are shown in Table 1. Distributions of *S. aureus* carriers and non-carriers stratified by population characteristics are shown in Table 2. A total of 632 participants were recruited, including 452 IP (71.5%) and 180 HS (28.5%). *S. aureus* was isolated from 105/632 (16.6%) of the study population. The *S. aureus* carrier prevalence in the two

Table 1
Demographic characteristics of inpatients (IP) and hospital staff (HS) in the Child Health Department (CHD) and Surgical Department (SD) at Korle Bu Teaching Hospital, Ghana, 2011–2012.

Characteristic	Level	IP (*N* = 452)	HS (*N* = 180)	*P*-value[a]
Sex [*n* (%[b])]	Female	180 (56)	144 (44)	<0.001
	Male	272 (88)	36 (12)	
Department [*n* (%[b])]	SD	203 (77)	61 (23)	0.01
	CHD	249 (68)	119 (32)	
Age group [*n* (%[b])]	≤14 years	252 (100)	0 (0)	<0.001
	>14 years	200 (53)	180 (47)	
Patient underlying diagnosis [*n* (%[c])]	Surgical	278 (61.5)		–
	Non-surgical	174 (38.5)		
Duration of hospitalisation [*n* (%[c])]	≤7 days	294 (65.0)		–
	>7 days	158 (35.0)		

[a] Difference in characteristic between IP and HS as assessed by χ^2 statistic.
[b] Percentage of IP and HS with a particular characteristic among all patients with that characteristic.
[c] Percentage of IP with that characteristic among all IP.

Table 2
Distribution of *Staphylococcus aureus* carriers and non-carriers stratified by department, sex, patient underlying diagnosis, status, duration of hospitalisation and age group at Korle Bu Teaching Hospital, Ghana, 2011–2012.

Stratifier	Group	*n* (%[a])	
		Carriers (*N* = 105)	Non-carriers (*N* = 527)
Sex	Female	58 (18)	266 (82)
	Male	47 (15)	261 (85)
Department	CHD	67 (18)	301 (82)
	SD	38 (14)	226 (86)
Age group	≤14 years	38 (15)	214 (85)
	>14 years	67 (18)	313 (82)
Status	IP	63 (14)	389 (86)
	HS	42 (23)	138 (77)
Patient underlying diagnosis	Surgical	38 (14)	240 (86)
	Non-surgical	25 (14)	149 (86)
Duration of hospitalisation	≤7 days	51 (17)	243 (83)
	>7 days	12 (8)	146 (92)

CHD, Child Health Department; SD, Surgical Department; IP, inpatients; HS, hospital staff.
[a] Percentage of carriers and non-carriers in specific stratum.

groups was 63/452 (13.9%) in IP and 42/180 (23.3%) in HS. HS had a higher chance of being *S. aureus* carriers than IP [odds ratio (OR) = 1.9, 95% confidence interval (CI) 1.2–2.9; *P* = 0.005]. IP staying ≤7 days in hospital were found to have a higher chance of being *S. aureus* carriers than those staying in the hospital for longer periods (OR = 2.5, 95% CI 1.3–4.8; *P* = 0.007).

3.2. Antimicrobial susceptibility

The highest prevalence of antimicrobial resistance was observed for penicillin (93%), tetracycline (28%) and fusidic acid (12%). The percentages of resistance were <5% for erythromycin, clindamycin, SXT, norfloxacin, gentamicin, rifampicin and mupirocin (Table 3). Resistance to linezolid and glycopeptides was not detected. Isolates obtained from IP tended to be more resistant than isolates from HS; tetracycline resistance was found to be 33% and 19% among IP and HS, respectively; resistance to fusidic acid was 17% in IP and 5% in HS (Table 3).

Of the 105 *S. aureus* isolates, 99 (94.3%) were MSSA and the remaining 6 isolates (5.7%) were resistant to cefoxitin and confirmed to be MRSA by *mecA* PCR. All MRSA were isolated from IP, leading to a carriage prevalence of 1.3% (6/452) in this group and an MRSA prevalence of ca. 10% (6/63) among *S. aureus* isolates from IP.

Table 3
Antibiotic resistance of *Staphylococcus aureus* isolates from inpatients (IP) and hospital staff (HS) at Korle Bu Teaching Hospital, Ghana, 2011–2012.

Antibiotic	*n* (%)		
	IP (*N* = 63)	HS (*N* = 42)	Total (*N* = 105)
Penicillin	62 (98)	36 (86)	98 (93)
Tetracycline	21 (33)	8 (19)	29 (28)
Fusidic acid	11 (17)	2 (5)	13 (12)
Cefoxitin	6 (10)	0 (0)	6 (6)
SXT	3 (5)	0 (0)	3 (3)
Erythromycin	4 (6)	1 (2)	5 (5)
Clindamycin	3 (5)	0 (0)	3 (3)
Norfloxacin	2 (3)	0 (0)	2 (2)
Gentamicin	1 (2)	1 (2)	2 (2)
Rifampicin	1 (2)	0 (0)	1 (1)
Mupirocin	0 (0)	1 (2)	1 (1)
Linezolid	0 (0)	0 (0)	0 (0)

SXT, trimethoprim/sulfamethoxazole.

Of the 105 *S. aureus* isolates, 14 (13.3%) were MDR. IP had a higher chance of MDR carriage compared with HS (OR = 2.4, 95% CI 1.5–460; *P* = 0.01). MDR carriage was not associated with sex, patient underlying diagnosis, department or DOH. The genotypes and resistance profiles of MDR *S. aureus* isolates are shown in Table 4 along with information on the IP they were isolated from.

3.3. Genetic diversity

A total of 55 *spa* types were found among the 105 isolates. The most frequent *spa* types were t084 (10%), t355 (10%), t127 (5.7%), t002 (3.8%), t537 (3.8%), t008 (2.9%), t304 (2.9%), t630 (2.9%), t861 (2.9%), t1510 (2.9%), t10821 (2.9%), t314 (1.9%), t363 (1.9%), t701 (1.9%), t1476 (1.9%), t2649 (1.9%), t2700 (1.9%) and t10822 (1.9%); the remaining 37 *spa* types were singletons. A minimum spanning tree displaying the relationships between the different *spa* types is illustrated in Fig. 1.

Among the six MRSA isolates, three from the SD and one from the CHD were ST88–SCC*mec* IV. Three of these four MRSA isolates displayed the same susceptibility profile, and two of them had the same *spa* type (Table 4). The two remaining isolates from the CHD

Table 4
Characteristics of multidrug-resistant *Staphylococcus aureus* among inpatients (IP) and hospital staff (HS) at Korle Bu Teaching Hospital, Ghana, 2011–2012.

spa type	CC	MLST	SCC*mec*	PVL	Antibiotype	Department	Status	Age (years)	Sex	DOH (days)	PUD	Antibiotic treatment
MRSA												
t537	CC1	ST72	V	–	PEN, FOX, TET	CHD	IP	1	F	5	Non-surgical	N/D
t064	CC8	ST8	V	–	PEN, FOX, NOR, TET, SXT	CHD	IP	6	M	2	Surgical	N/D
t325	CC88	ST88	IV	–	PEN, FOX	CHD	IP	5	M	1	Surgical	Ceftriaxone and cefuroxime
t2649	CC88	ST88	IV	–	PEN, FOX, TET	SD	IP	21	M	45	Surgical	Cloxacillin
t2649	CC88	ST88	IV	–	PEN, FOX, TET	SD	IP	21	M	1	Surgical	Cloxacillin
t1951	CC88	ST88	IV	–	PEN, FOX, TET	SD	IP	27	M	1	Surgical	N/D
MSSA												
t10808	N/D	N/D	N/A	–	PEN, TET, SXT	CHD	IP	39	M	1	Surgical	N/D
t127	N/D	N/D	N/A	–	PEN, FUS, SXT, NOR	CHD	IP	4	M	2	Surgical	Cefuroxime
t304	N/D	N/D	N/A	–	PEN, FUS, NOR	CHD	IP	65	M	1	Surgical	Ceftriaxone
t2700	N/D	N/D	N/A	–	PEN, FUS, CLI, ERY	CHD	IP	13	F	20	Non-surgical	N/D
t630	N/D	N/D	N/A	–	PEN, TET, FUS	CHD	IP	17	M	10	Non-surgical	Ceftriaxone and cefuroxime
t4805	N/D	N/D	N/A	–	PEN, MUP, GEN	CHD	HS	51	F	N/A	N/A	N/D
t314	N/D	N/D	N/A	+	PEN, TET, FUS	SD	IP	70	M	1	Surgical	N/D
t355	N/D	N/D	N/A	–	PEN, TET, FUS	CHD	IP	79	M	5	on surgical	No antibiotic

MLST, multilocus sequence type; CC, clonal complex; SCC*mec*, staphylococcal chromosome cassette *mec*; PVL, Panton–Valentine leukocidin; DOH, duration of hospitalisation (at the time of screening); PUD, patient underlying diagnosis; MRSA, meticillin-resistant *S. aureus*; MSSA, meticillin-susceptible *S. aureus*; N/D, not determined; N/A, not applicable; PEN, penicillin; FOX, cefoxitin; TET, tetracycline; NOR, norfloxacin; SXT, trimethoprim/sulfamethoxazole; FUS, fusidic acid; CLI, clindamycin; ERY, erythromycin; MUP, mupirocin; GEN, gentamicin; CHD, Child Health Department; SD, Surgical Department.

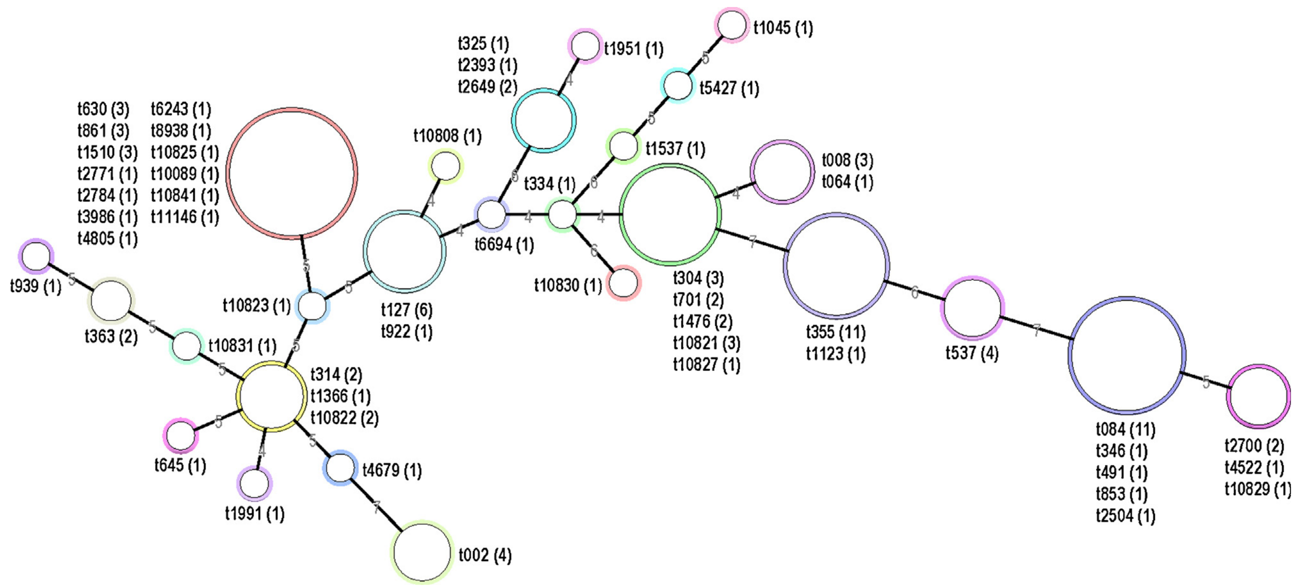

Fig. 1. Minimum spanning tree displaying the relationships between the different *spa* types. *spa* types were assigned to the same complex if two neighbouring *spa* types did not differ by more than three changes. Complexes are displayed as coloured circles and the size of each circle indicates the number of isolates within the complex. *spa* types and the number of isolates displaying a particular *spa* type are shown. Complexes that differ by more than three changes are connected by lines, and the length of each line indicates the number of changes.

belonged to ST8–SCC*mec* V and ST72–SCC*mec* V. All MRSA isolates were negative for PVL, whereas a high percentage (23%) of PVL-positive isolates was observed among MSSA.

3.4. Antimicrobial usage

History of antimicrobial usage retrieved from 198 (43.8%) records of IP showed that 20%, 16% and 13% of IP had received cefuroxime, ciprofloxacin and ceftriaxone, respectively.

4. Discussion

This is the first baseline study that provides insight into the prevalence of nasal carriage, antimicrobial susceptibility and clonal structure of *S. aureus* and MRSA among IP and HS in Ghana. The study reveals a statistically significant lower *S. aureus* carrier prevalence among IP (13.9%) compared with HS (23.3%). The difference in *S. aureus* carrier prevalence among IP and HS could be due to the observation that ≥44% of the IP received antibiotic therapy at the time of sampling. In line with this hypothesis, *S. aureus* carriage was less frequent among IP with a long DOH (8%) compared with IP with shorter stays (17%).

Previous studies have reported an association of age and sex with *S. aureus* carriage [18], but this study only found an association between nasal carriage of *S. aureus* with being a HS, which is in agreement with a recent study in Gabon [8]. The overall MRSA carrier prevalence of 0.9% (6/632) observed in this study is comparable with those reported by previous carriage studies in African hospitals: 0.0% (0/496) in Sudan [6]; 0.7% (1/145) in Somalia [7]; and 1.4% (4/295) in Gabon [8].

MRSA was recovered from six IP with various surgical conditions. The finding of four IP carrying MRSA ST88–IV was not surprising as this clone has been frequently reported in communities and hospitals in major African cities [8–10]. Two IP who stayed in the same ward 4 months apart carried isolates with the same *spa* type (t2649) and antibiogram (Table 4). However, there was no direct evidence of transmission of this MRSA lineage between the IP and from HS to IP. Possible environmental sources were not investigated in the study. It is of interest to note that two of the MRSA were isolated from IP receiving cefuroxime, ceftriaxone and cloxacillin. Routine nasal screening of IP, especially surgical patients, which is currently not performed in the health facility, could be considered to prevent treatment failure to further ameliorate morbidity, mortality and cost of stay at the hospital. In addition, active surveillance has been shown to minimise MRSA transmission to other patients and hospital staff [19].

The relatively high prevalence of resistance to fusidic acid, especially among IP (17%), is surprising, although of limited clinical relevance since fusidic acid is not frequently used in Ghana. This resistance pattern is in contrast to studies in South Africa where full susceptibility to fusidic acid was observed [20,21]. The genetic background of fusidic acid resistance was not investigated in this study and the reason for the high prevalence of resistance observed in the absence of any apparent selective pressure remains unknown. The occurrence of mobile *fus* genes has been reported in the USA where fusidic acid is not available in the market [22]. Further studies are needed to throw light on possible co-selecting factors favouring the spread of fusidic acid resistance in the absence of selective pressure.

The high prevalence of resistance to penicillin and tetracycline is comparable with previous reports from other African studies [6,7,11] and may reflect the frequent usage of these antibiotics in the community in Ghana, where they can be purchased over the counter without prescription. However, this finding has limited clinical relevance since penicillin and tetracycline are not routinely used for treatment of staphylococcal infections. According to the Standard Treatment Guidelines in Ghana, flucloxacillin should be the drug of choice for the treatment of staphylococcal infections. The observed high rate of susceptibility to vancomycin, teicoplanin and linezolid indicates that these agents remain excellent reserve drugs for the treatment of severe *S. aureus* infections in Ghana. Even though the resistance trends for norfloxacin, clindamycin, erythromycin, SXT, gentamicin and rifampicin were low (<5%), prudent usage of these antimicrobial agents should be encouraged to prevent selection of resistance of *S. aureus* to these drugs.

A high genetic diversity was observed among the *S. aureus* isolates, with t084 and t355 being the most prevalent *spa* types. *S. aureus* with *spa* type t084 has been reported frequently in healthy human nasal isolates elsewhere [4]. Noteworthy, PVL-positive MSSA belonging to t355 associated with ST152 was abundant in this study and has been recognised in communities and hospitals in West Africa [23,24] and as a community-acquired MRSA in Central Europe [25].

ST88 was the most common MRSA lineage and appears to be one of the predominant MRSA clones in Africa as it was previously reported as a major MRSA clone in Nigeria, Mali, Gabon and other African countries [8–10,24]. In contrast, this MRSA lineage is uncommon in Europe [9,26,27]. Among the other MRSA isolated from Korle Bu Teaching Hospital in Ghana, ST8 has also been found in Cameroon and Madagascar; it has also been reported to be one of the major epidemic clones in Nigeria [9,10]. ST72 has been reported in Nigeria and Gabon as an MSSA clone [10,28], but only as major MRSA clone from communities in Australia and South Korea [29,30].

In conclusion, this study provides the first baseline epidemiological information on the prevalence of nasal carriage and genetic diversity of *S. aureus* and MRSA among IP and HS at a major referral hospital in Ghana. The results indicate a low frequency of MRSA carriage, high genetic diversity among *S. aureus* isolates, and a higher risk of nasal carriage of MDR *S. aureus* among IP compared with HS. The finding of MRSA among surgical patients suggests that screening of IP before surgery, which is not a routine practice in the hospital, may be an important infection control measure to be implemented in the future.

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Ethical approval

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

Competing interests

None declared.

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