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Molecular typing of antibiotic-resistant *Staphylococcus aureus* in Nigeria

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Summary
Background: Antibiotic-resistant *Staphylococcus aureus* including methicillin-resistant strains (MRSA) are a major concern in densely populated urban areas. Initial studies of *S. aureus* in Nigeria indicated existence of antibiotic-resistant *S. aureus* strains in clinical and community settings.
Methods: 73 biological samples (40 throat, 23 nasal, 10 wound) were collected from patients and healthcare workers in three populations in Nigeria: Lagos University Teaching Hospital, Nigerian Institute of Medical Research, and Owerri General Hospital.
Results: *S. aureus* was isolated from 38 of 73 samples (52%). Of the 38 *S. aureus* samples, 9 (24%) carried the Panton-Valentine leukocidin gene (PVL) while 16 (42%) possessed methicillin resistance genes (*mec*A). Antibiotic susceptibility profiles indicated resistance to several broad-spectrum antibiotics.
Conclusion: Antibiotic-resistant *S. aureus* isolates were recovered from clinical and community settings in Nigeria. Insight about *S. aureus* in Nigeria may be used to

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improve antibiotic prescription methods and minimize the spread of antibiotic-resistant organisms in highly populated urban communities similar to Lagos, Nigeria.

Introduction

Staphylococcus aureus (*S. aureus*) is a gram-positive bacterium that exists in clinical and community settings across the world [1—3]. Recent estimates suggest approximately 30% of humans are asymptomatic carriers of *S. aureus* in their nares and/or throat [4]. Carriers of *S. aureus* are between three to six times more likely to suffer from a clinical *S. aureus* infection than non-carriers [4,5]. Furthermore, about 80% of invasive *S. aureus* infections originate from a strain occurring in the host's natural microflora [3,4]. Clinical infections of *S. aureus* can range from mild to severe, leading to various diseases such as skin and deep tissue infections, pneumonia, and osteomyelitis [4].

In hospital settings, antibiotic-resistant *S. aureus* is a common etiological agent of healthcare-associated infections (HAIs) [6]. Highly virulent antibiotic-resistant *S. aureus* in these settings is of particular concern, including methicillin-resistant strains. In the United States alone, approximately 95,000 invasive methicillin-resistant *S. aureus* (MRSA) infections occur each year [5]. The *mec*A gene found in methicillin-resistant *S. aureus* organisms confers resistance against a host of antibiotics including methicillin and oxacillin; this genetic property may cause these organisms to exhibit resistant to other antibiotics including tetracyclines, penicillins, and carbapenems. The PVL genes encode the Panton-Valentine leukocidin, a putative virulence factor, which is hypothesized to enhance the bacterium's ability to cause severe infections in human and animal hosts [7,8].

Healthcare-associated (HAI) and community-associated (CA) *S. aureus* are major global health problems, yet little is known about their impact in Nigeria. Nigeria is the most populous country in Africa and boasts the second most populous city in Africa, Lagos, which has a population over 13 million people [9]. Due to a high population density (approximately 7000 people per square mile) [10], sufficient medical attention is often not available to all patients and significant public health problems are often poorly understood. Specifically, antibiotic resistance profiles of *S. aureus* isolates may be conferred from wide spread misuse of these antibiotics. In a prior study of *S. aureus* in Nigeria, 68 *S. aureus* samples were collected in various health institutions throughout the country [1]. Eleven isolates (11/68, 16%) were identified as MRSA. Remaining isolates showed resistance to such antibiotics as erythromycin, tetracycline, and TMP/SMX.

Researchers from the Center for Emerging Infectious Diseases (CEID) at the University of Iowa collaborated with researchers at the Nigerian Institute of Medical Research in Lagos, Nigeria. The objective of the research was to identify molecular types of antibiotic-resistant *S. aureus* within community and clinical settings in Lagos and Owerri, Nigeria.

Methods/materials

Fifty-eight nasal or throat samples were collected from patients, healthcare workers, or volunteers at Lagos University Teaching Hospital Dental Clinic (LUTH-DC) and Nigerian Institute of Medical Research HIV Clinic Yaba, Lagos (NIMR-HC). Nasal and throat samples were collected from HIV positive volunteers, and from other NIMR Volunteers, while throat samples were collected from patients and healthcare workers at the LUTH-DC. In total, 58 biological samples were collected from 37 participants in June and July 2012. Institutional Review Board approval was obtained through the University of Iowa in Iowa City, USA, Nigerian Institute of Medical Research Yaba, Lagos, Nigeria, and Dr. Adesola Kehinde Umeizudike, Department of Preventive Dentistry, Lagos University Teaching Hospital (LUTH, Idi-Araba) in Lagos, Nigeria. Informed consent was obtained from participants in Lagos, Nigeria. Additionally, fifteen *S. aureus* stock isolates from Owerri General Hospital in Owerri, Nigeria (eight wound and five throat samples) and Lagos University Teaching Hospital in Lagos, Nigeria (two wound isolates) were included with the collected samples for molecular analysis (Table 1).

The nose and throat samples were collected using sterile cotton swabs. Once collected, each swab was suspended in sterile 6.5% Nutrient Broth No. 2 (NB), in tubes, and incubated at 37 °C for 24 h [11]. Ten microliters was transferred from each incubated tube to plate of *Staphylococcus* medium No. 110 (SM) and allowed to grow at 37 °C for 48 h [12]. After incubation, samples displaying orange, circular colonies on SM were sub-cultured

Table 1 Sample collection location and body sites.

Location	Nasal	Throat	Wound	Total samples	*S. aureus* isolates	% Positive	95% CI	MRSA isolates	% Positive	95% CI
LUTH	0	12	0	12	6	50.0	21.7–78.3	1	8.3	0.0–24.0
Dental clinic										
NIMR	13	13	0	26	14	53.8	34.7–73.0	8	30.8	13.0–48.5
HIV volunteers										
NIMR	10	10	0	20	10	50.0	28.1–71.9	2	10.0	0.0–23.1
Other volunteers										
Owerri hospital	0	5	8	13	8	61.5	35.1–88.0	5	38.5	12.0–64.9
LUTH	0	0	2	2	0	—	—	0	—	—
Medical center										
Total	23	40	10	73	38	52.1	40.1–63.5	16	21.9	12.4–31.4

Note—Location: LUTH = Lagos University Teaching Hospital, NIMR = Nigerian Institute of Medical Research.

on Mueller Hinton Agar (MHA) and incubated for another 24 h. Presumptive *S. aureus* isolates were confirmed by Gram stain, catalase test, and coagulase test. Positive isolates were shipped to the Center for Emerging Infectious Diseases (CEID) for additional diagnostic confirmation and molecular testing [13].

Positive *S. aureus* isolates were subject to polymerase chain reaction (PCR) for *S. aureus* protein A (*spa*) using primers described by Ridom GmbH (Würzburg, Germany, 2004; http://www.ridom.de/doc/Ridom_spa_sequencing.pdf), methicillin resistance (*mec*A) gene, and presence of PVL gene [14,15]. Isolates with antibiotic-resistant genes, or a common *spa* type, underwent multilocus sequence typing (MLST) [16]. Lastly, presumptive *S. aureus* isolates were subjected to antibiotic susceptibility testing by broth microdilution technique, defined by Weigand and colleagues [17], using the following antibiotics: Oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim–sulfamethoxazole (TMP/SMX), gentamycin, levofloxacin, vancomycin, daptomycin, quinupristin/dalfopristin, linezolid, and rifampin. *S. aureus* isolates were stored in glycerol solution at −80 °C, until needed [13].

Results

Overall, 73 samples (40 throat, 23 nasal, 10 wound) were analyzed as part of the study; 58 samples were collected from participants in Lagos, Nigeria while the remaining 13 samples included previously collected stock isolates from participants in Lagos and Owerri, Nigeria. *S. aureus* was isolated from 38 of 73 samples (52%; 95% CI 40.6%–63.5%).

The overall prevalence of *S. aureus* colonization among healthcare workers and patients was 52% (30/58; 95% CI 38.9%–64.6%). The additional fifteen stock isolates from Owerri General Hospital (8 wound samples and 5 throat samples) and Lagos University Teaching Hospital (2 wound samples) were included in molecular analysis but not as part of colonization rates because they were collected and identified as *S. aureus* isolates in a previous study. Fifty percent of samples (6/12; 95% CI 21.7%–78.3%) collected from Lagos University Teaching Hospital Dental Clinic were confirmed *S. aureus*, 54% (14/26; 95% CI 34.5%–73.0%) from Nigerian Institute of Medical Research (NIMR) HIV volunteers were confirmed *S. aureus*, and 50% (10/20; 95% CI 28.1%–71.9%) from other NIMR Volunteers were confirmed *S. aureus*. In total, 19% of colonization samples (11/58; 95% CI 8.9%–29.1%) and 22% of all samples, including

colonization samples and stock isolates (16/73; 95% CI 12.4%—31.4%), were *mec*A positive.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out on the 38 *S. aureus* isolates. Thirty-two (84%; 95% CI 72.6%—95.8%) were resistant to tetracycline, 29 (76%; 95% CI 62.8%—89.8%) were resistant to TMP/SMX, 7 (18%; 95% CI 6.1%—30.7%) were resistant to levofloxacin, 5 (13%; 95% CI 2.4%—23.9%) were resistant to gentamycin, and 5 (13%; 95% CI 2.4%—23.9%) were resistant to erythromycin. All isolates were susceptible to clindamycin, vancomycin, daptomycin, Q/D, linezolid, and rifampin. Furthermore, 16 (42%; 95% CI 26.4%—57.8%) of isolates were found to be phenotypically methicillin-resistant—only one *S. aureus* isolate with the *mec*A gene (1/16) was susceptible to oxacillin. Only one oxacillin-resistant *S. aureus* isolate did not possess the *mec*A gene.

Molecular analysis

PCR was conducted on presumptive *S. aureus* isolates to confirm *spa*, *mec*A, and PVL status. Thirteen distinct *spa* types were identified among the 38 *S. aureus* isolates taken from wound, throat, or nasal sites. The *spa* types common to more than one individual were t064 (11/38, 29%; 95% CI 14.5%—43.4%), t355 (10/38, 26%; 95% CI 12.3%—40.3%), t084, t091, and t657 which accounted for three isolates apiece, or 8% of all isolates, respectively. PVL-encoding genes were identified in 24% (9/38; 95% CI 10.2%—37.2%) of known *S. aureus* isolates, with all but one found in methicillin-susceptible *S. aureus* (MSSA); all nine isolates carrying the PVL genes were *spa* type t355.

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed on eight *S. aureus* isolates, six of which had the *mec*A gene—five isolates were ST8 (*spa* types t064, t5604, t11754) and one of each was ST152 (*spa* type t355), ST772 (*spa* type t657), ST14 (*spa* type t084).

Discussion

Based on results of this study, we have reasons to suggest that antibiotic-resistant *S. aureus*, specifically methicillin-resistant strains, and potentially virulent *S. aureus* strains exist in clinical and community settings in Nigeria. In agreement with previous studies, we found t064/ST8 as the most common *spa* type among the study population. Furthermore, researchers are of the view that t064 is the most common *spa* type among HIV-positive patients in Nigeria [18]. Similarly, t064 was the most frequent *spa* type among participants in our study (11/38, 29%) including 44% (4/9) of HIV-positive participants exhibiting nose or throat colonization, often times in both sites. Shittu and colleagues [1] primarily collected wound samples from individuals suffering from a clinical *S. aureus* infection whereas our samples were primarily collected from nose and throat sites of clinically healthy individuals with additional analysis of *S. aureus* wound isolates from a recent study. Still, similar *spa* types (e.g. t064, t355) were found in both epidemiologic studies suggesting that colonized individuals may facilitate transmission, especially among immune-compromised individuals. Conducting a longitudinal epidemiologic study among these populations would be useful to determine if nasal and throat colonization is transient and whether or not individuals colonized with *S. aureus* can be infected with those same organisms.

Sequence type (ST) 8 (e.g. *spa* type t064, CA-MRSA) has been documented to cause severe infections in humans and can be found within animals and humans in Europe, Asia, and the Americas, as well as Africa [5,18—21]. ST8 (*spa* type t064) with *mec*A gene was isolated from participants in all three populations that we sampled and represented two distinct geographical regions of Nigeria which are separated by more than 300 miles. Furthermore, ST8 was isolated from nose, throat, and wound sites and from both HIV-positive and non HIV-positive individuals. These results tend to suggest that antibiotic-resistant *S. aureus*, specifically ST8, is ubiquitous in various geographic regions of Nigeria.

Our results also showed that 30 individuals (52%) were positive for *S. aureus* colonization in either their nose or throat (Table 2). Still, we are aware that because our study population includes both HIV-positive and non HIV-positive individuals, it may be difficult to draw conclusions about *S. aureus* colonization rates among the general population in Nigeria. Evidence suggests *S. aureus* colonization rates and MRSA clinical infections may be markedly different between HIV-positive populations and HIV-negative populations [22—24]. Researchers [18] found that 33% of HIV-positive participants (124/375) were colonized with *S. aureus*, twenty of whom were colonized with MRSA, compared to 21% of HIV-negative participants (78/370) colonized with *S. aureus*.

In our study, 69% of individuals (9/13) from the NIMR HIV clinic were colonized with *S. aureus*

Table 2 Molecular epidemiology of *S. aureus* from 30 participants.

ID	Participant	Population	Site	*spa* type	PVL	*mecA*	MLST	Resistance profile
1	HCW	A	T	t084	–	–	NT	Tet
2	Patient	A	T	t084	–	–	NT	None
3	HCW	A	T	t127	–	–	NT	Tet
4	Patient	A	T	t084	–	–	NT	O, Tet, TMP
5	HCW	A	T	t064	–	+	ST8	O, Tet, TMP
6	HCW	A	T	t385	–	–	NT	Tet
7a	Patient	B	N	t657	–	+	NT	O, E, TMP, G, L
7b	Patient	B	T	t657	–	+	NT	O, E, TMP, G, L
8	Patient	B	N	t064	–	+	NT	O, Tet, TMP
9a	Patient	B	N	t064	–	–	NT	Tet, TMP
9b	Patient	B	T	t657	–	+	ST772	O, E, TMP, G, L
10	Patient	B	N	t064	–	+	NT	O, Tet, TMP
11	Patient	B	T	t064	–	+	ST8	O, Tet, TMP
12a	Patient	B	N	t355	+	–	NT	Tet, TMP
12b	Patient	B	T	t355	+	–	NT	Tet, TMP
13a	Patient	B	N	t355	+	–	NT	Tet, TMP
13b	Patient	B	T	t355	+	–	NT	Tet, TMP
14a	Patient	B	N	t064	–	+	NT	O, Tet, TMP
14b	Patient	B	T	t355	+	–	NT	Tet, TMP
15	Patient	B	N	t355	+	+	ST152	Tet, TMP
16	Volunteer	C	N	t064	–	+	ST8	O, Tet, TMP
17a	Volunteer	C	N	t355	+	–	NT	Tet, TMP
17b	Volunteer	C	T	t2078	–	–	NT	None
18	Volunteer	C	N	t355	+	–	NT	Tet, TMP
19	Volunteer	C	T	t064	–	+	NT	O, Tet, TMP
20	Volunteer	C	N	t355	+	–	NT	Tet, TMP
21a	Volunteer	C	N	t091	–	–	NT	Tet, TMP, L
21b	Volunteer	C	T	t091	–	–	NT	Tet, L
22a	Volunteer	C	N	t5064	–	–	NT	Tet, L
22b	Volunteer	C	T	t2216	–	–	NT	Tet
23	Patient	D	T	t1452	–	–	NT	Tet, E, TMP
24	Patient	D	T	t064	–	+	NT	O, Tet, TMP, G
25	Patient	D	W	t5604	–	+	ST8	O, Tet, TMP
26	Patient	D	W	t11754	–	–	ST8	Tet, E, TMP, G, L
27	Patient	D	W	t064	–	+	NT	O, Tet, TMP
28	Patient	D	W	t064	–	+	NT	O, Tet, TMP
29	Patient	D	W	t159	–	–	NT	TMP
30	Patient	D	W	t064	–	+	NT	O, Tet, TMP

Note—Participant type: HCW = healthcare worker; population: A = dental clinic, B = HIV clinic, C = NIMR volunteers, D = Owerri hospital; Site: N = nose, T = throat, W = wound; MLST: NT = not tested; resistance profile: O = oxacillin, Tet = tetracycline, E = erythromycin, TMP-SMX = TMP, G = gentamycin, L = levofloxacin.

with five of the nine individuals colonized in both nose and throat. It seems that immunodeficiency facilitates antibiotic-resistant *S. aureus* transmission, since our findings and those from other researchers [1,18,22–24] indicate higher colonization and infection rates of *S. aureus* among HIV-positive individuals, compared to their healthy counterparts. HIV clinics are often crowded, which may facilitate transmission between individuals. It should be pointed out that HIV-positive individuals who are colonized with *S. aureus* may be at increased risk of developing an infection in the community or hospital. Additional investigation should be conducted to determine *S. aureus* colonization and infection rates among HIV-positive individuals and non HIV-positive individuals in various clinical settings.

Antibiotic resistance profiles of the isolates collected for this study are consistent with earlier studies investigating *S. aureus* in Nigeria [1]. We found antibiotic-susceptibility profiles similar to other researchers in Nigeria including resistance to oxacillin, tetracycline, and TMP/SMX; none of the tested *S. aureus* isolates in this study exhibited resistance to vancomycin, linezolid, daptomycin, or rifampin. Tetracycline and

TMP/SMX are among the most commonly used antibiotics in Nigeria because these antibiotics are cheap and easily obtainable through lenient medication regulations. Conversely, more expensive and less frequently used antibiotics like linezolid and vancomycin were not readily resisted. Based on both findings, it could be concluded that antibiotic over-prescription and misuse, specifically widespread distribution of broad-spectrum antibiotics (tetracycline and TMP/SMX), may elevate antibiotic-resistant *S. aureus* nasal and pharynx carriage, transmission, and subsequent infection, especially in urban populations of Nigeria.

Regarding virulence factors, previous findings indicated 40% (23/57) of methicillin-susceptible *S. aureus* (MSSA) isolates were PVL-positive; no PVL-positive MRSA was reported in the study [1]. In agreement with their report, we found PVL-positive isolates most often in MSSA. A single PVL-positive MRSA organism was isolated in our study, suggesting that PVL-encoding genes exist in antibiotic-resistant organisms in Nigeria. These antibiotic-resistant and potentially virulent organisms may cause more severe infections in humans and survive through antibiotic treatment.

Intervention strategies to reduce *S. aureus* carriage in the healthcare setting have been well-studied. For example, a report written by Friedel and Climo [25] indicated topical intranasal antimicrobials such as Mupirocin are effective in reducing *S. aureus* nasal carriage but have been associated with antimicrobial resistance and may be ineffective for long-term decolonization of *S. aureus*. Adesida and colleagues [26] suggest *S. aureus* colonization rates among medical students at a tertiary medical center in Lagos, Nigeria are heavily influenced by personal behaviors. They concluded adhering to personal hygiene behaviors including handwashing will likely decrease colonization rates among healthcare personnel and could be effective as a primary infection control measure in Nigeria [26]. Still, the key to a successful infection control intervention is addressing a combination of hand hygiene determinants at the individual, interpersonal, and institutional levels [27].

It is pertinent to point out that the present study suffers some limitations. First, the sample size was small with only 73 samples, 58 of which were collected from participants as part of this study. As a result, we are careful not to conclude that *S. aureus* colonization rates from our sample collection are indicative of the true colonization rate of residents in Lagos, Nigeria. Furthermore, our samples were collected conveniently, which may suggest that the samples may not be representative of the entire population of Lagos, Nigeria. The samples were collected from patients and healthcare workers who were readily available and willing to participate in the study because there was a short time frame on site in Lagos to collect and analyze each sample. Also, study participants were from the Nigerian Institute of Medical Research and Lagos University Teaching Hospital located less than two miles apart. Consequently, *S. aureus* colonization rates and *spa* types found in this study may not be reflective of the situation in other parts of Lagos, Nigeria.

Still, there were advantages to this observational study. For instance, we conducted extensive molecular typing on collected isolates. PCR was performed to identify *spa*, *mec*A, and PVL genes for all *S. aureus* isolates. Also, each presumptive *S. aureus* isolate was tested for susceptibility against a wide range of antibiotics used in Nigeria so we can be certain that *S. aureus* isolates in this study exhibit genotypic and phenotypic antibiotic resistance against commonly used medications in Lagos, Nigeria. Therefore, it can be suggested that antibiotic prescription methods in urban environments similar to Lagos affect antibiotic-resistant profiles of *S. aureus* organisms.

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Competing interests

None declared.

Ethical approval

Not required.

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