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CARRIAGE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENETIC DIVERSITY OF *STAPHYLOCOCCUS AUREUS* AND MRSA ISOLATES RECOVERED FROM STUDENTS IN A KENYAN UNIVERSITY
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CARRIAGE, ANTIMICROBIAL SUSCEPTIBILITY PROFILES AND GENETIC DIVERSITY OF *STAPHYLOCOCCUS AUREUS* AND MRSA ISOLATES RECOVERED FROM STUDENTS IN A KENYAN UNIVERSITY

O. Y. Khasabuli, C. Ngugi and J. Kiiru

ABSTRACT

Objective: **To determine the carriage, antimicrobial susceptibility profiles and genetic diversity of *Staphylococcus aureus* and MRSA isolates recovered from students in a Kenyan University.**

Study design: **A cross sectional descriptive study.**

Setting: Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya.

Subjects: **A total of 237 healthy students residing within the University residence halls were recruited.**

Results: **A total of 231 *S. aureus* isolates were recovered. All isolates were susceptible to nitrofurantoin and linezolid and resistant in high numbers (194, 81.9%) to ampicillin. Resistances to amoxicillin-clavulanic acid, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin and trimethoprim-sulfamethoxazole were below 20%. The overall prevalence of MRSA among the study population was 11.3% (26/231). Based on carriage of the *mec* cassettes, the SCC*mec*V (61.5%) was more prevalent among the MRSA followed by SCC*mecII* (53.9%). Carriage of the *lukFS-PV* gene was 26.9% and 35.7% among the MRSA and MSSA strains respectively, and there was no statistical association between the two strains with regard to carriage of the gene (p=0.487). Analysis of genetic relations showed evidence of strain sharing among students.**

Conclusion: **The study revealed the presence of MRSA strains, which are also multi-drug resistant, circulating among a healthy student population in a university setting within Central Kenya. Therefore, these results indicate the existence of potential risk factors, thus necessitating a comprehensive surveillance on MRSA and studies on control measures to help in curbing the spread of MRSA strains.**

INTRODUCTION

Staphylococcus aureus is found on the skin surface and the nostrils of approximately 30% of healthy people (1). Although recognised as a commensal, this bacterium can also cause human infections such as boils and pimples, septicemia and pneumonia. Of all colonisable surfaces, the nasal cavity is the primary "reservoir" although significant colonization has been reported in extra-nasal sites such as the vagina, skin, and the gastrointestinal tract (2). The usual transmission among humans is person-to-person transmission (3). Fomites such as phones have also been implicated in the spread of *S. aureus* (4,5).

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections occur in any setting, but several factors may facilitate the spread of MRSA. These include crowded living conditions, skin cuts, close skin-to-skin contact, contaminated inanimate surfaces, and lack of proper hygiene (6,7). MRSA continue to develop resistance towards available antibiotics, thus rendering management of infections caused by MRSA problematic (8).
Schoolmates are now considered a risky group for MRSA infections and Universities are ideal settings where spread of MRSA is facilitated by close contact among susceptible individuals (3). Naturally, students tend to live in close proximity and share a lot of material such as pens and phones. Such items could act as potential fomites for transmission. Students suffering from skin and other soft tissue infections may experience a lot of discomfort, stigmatization and often miss class days (9). Although a few studies have focused on college students or students participating in particular sporting activities such as football and athletics, no study has been done to determine the extent of colonization of MRSA among students in any of the Kenyan Universities. As such, the prevalence of MRSA and the occurrence of multi-drug resistant strains in this population remain unknown.

Presently, the frequency of incidence of infections caused by MRSA strains continues to grow in school settings worldwide (3,10). Thus, it is likely that Kenyan University students are also at a risk of MRSA infections. Due to unavailability of information on the prevalence of MRSA colonization among university students, management of MRSA infections among students may be ineffective. Prevalence reports are crucial towards implementation of strategies that can help in preventing the spread of MRSA among high risk groups, and perhaps prevent outbreaks of MRSA infections (11). In order to investigate the colonization of *Staphylococcus aureus*, we collected nasal swabs and swabs from phones and pens of students from a local university. The isolates were analysed for resistance to various antimicrobials, prevalence and genetic basis for the MRSA phenotype, carriage of PVL genes and genetic relatedness.

MATERIALS AND METHODS

Study Area and Study Population

The study was conducted at the main campus of Jomo Kenyatta University of Agriculture and Technology in Kenya, from October 2015 to July 2016. Students who resided within the university hostels and those who agreed to give a written consent for participation were recruited. Non-resident students, those on antibiotics and those who refused to give their written consent were not recruited.

Sampling size and sampling technique

A total of 237 participants were recruited. A systematic random sampling procedure was used to randomly select students from six residence halls. The number

of participants selected in each hall was based on a ratio of the total number of rooms in each hall.

Collection of samples

A sterile nasal swab softened using a drop of sterile normal saline was inserted into each nostril and gently rotated to scoop a specimen from nasal secretions. The swabs were labelled using codes unique to each participant. The swabs were also collected from phones and pens (fomites) from each of the recruited students. This was done by swabbing over surface of the fomite using a wet sterile swab. All swabs were transferred to Amies transport media before transportation to the laboratory for analysis within 4 hrs.

Bacterial isolation and identification

Upon arrival in the laboratory, the swabs were enriched into trypticase soy broth (TSB) and incubated for 18 to 24 hours. A 10µl aliquot from the enrichment was inoculated on Mannitol Salt Agar (Oxoid) plates and incubated aerobically at 35°C for 24 hours. Standard methods of identifying *Staphylococcus aureus* were based on colony morphology, Gram staining, coagulase and catalase tests. In order to confirm the isolates as *S. aureus*, *spa*-PCR typing was done. Pure cultures were prepared and preserved in trypticase soy broth supplemented with 15% glycerol and frozen at -20°C until further analysis.

Determination of antibiotic resistance patterns

The modified Kirby-Bauer disk diffusion method for antibiotic susceptibility testing was employed using commercial antimicrobial discs and results interpreted based on the CLSI guidelines. The discs included ampicillin (AMP, 10µg), amoxicillin-clavulanic acid (AMC, 30), ciprofloxacin (CIP, 10µg), erythromycin (E, 15µg), gentamicin (CN, 10µg), cefoxitin (FOX, 30µg), linezolid (LZD, 30µg), norfloxacin (NOR, 10µg), nitrofurantoin (F, 300µg), chloramphenicol (C, 30µg) and trimethoprim-sulfamethoxazole (SXT, 25µg). *Staphylococcus aureus* (MRSA) ATCC® 33591 was used as control for disc potency and to check the quality of the media. Identification of methicillin-resistant *S. aureus* and multidrug-resistant *S. aureus*

The MRSA strains were detected based on their susceptibility to cefoxitin. Strains with inhibition zone diameters of ≤21mm were considered as methicillin-resistant while those with inhibition zone diameters of ≥21 mm were considered as methicillin-susceptible. Multi-drug resistance was taken as resistance to any β-lactam and at least three antibiotics from the other classes.

Isolation and storage of DNA

DNA from the *S. aureus* strains was recovered using 10% Chelex solution prepared using 1X TE buffer. Isolated DNA was stored at -20°C for future use.

Screening for *MecA* gene and SCC*mec* typing

Amplification and detection of *mecA* gene and SCC*mec* elements was done on all the *S. aureus* isolates using the polymerase chain reaction (PCR) method. For the *mecA* gene, 4µl of the ready to mix 5x FIREPol®Master Mix (Solis Biodyne), was added to 0.4µl of each primer, 1µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA. The forward and reverse primers sequences were 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and 5'-AGT TCT GCA GTA CCG GAT TTG C-3' respectively. Amplification reactions were set at an initial

denaturation temperature of 95°C for 5 minutes. Thereafter, the preparation was subjected to 30 cycles of denaturation at 94°C for 60 seconds, annealing at 56°C for 60 seconds, extension at 72°C for 60 seconds and final extension at 72°C for 7 minutes. The expected Amplicon size was 533bp.

For SCC*mec*-typing, amplification of SCC*mec* elements was done using primers specific for SCC*mec*I, II, III, IV and V. In every PCR tube, 4µl of the ready to mix 5x FIREPol®Master Mix (Solis Biodyne), 0.4µl of both forward and reverse primers, 1µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA. Amplification reactions were initiated with a denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 seconds, 65°C for 45 seconds, and 72°C for 1.5 min and another 25 cycles of 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 1.5 min, ending with a final extension step at 72°C for 10 min. A known control strain was included as a positive control for each PCR set. The primer sequences for the SCC*mec* types were as follows: SCC*mec*I forward primer 5'- GCT TTA AAG AGT GTC GTT ACA GG-3' and reverse primer 5'-GTT CTC TCA TAG TAT GAC GTC C-3'= SCC*mec*II forward primer 5'- CGT TGA AGA TGA TGA AGC G-3' and reverse primer 5'-CGA AAT TGG TTA ATG GAC C-3'= SCC*mec*III forward primer 5'-CCA TAT TGT GTA CGA TGC G-3' and reverse primer 5'-CCT TAG TTG TCG TAA CAG ATC G-3'= SCC*mec*IVa forward primer 5'-GCC TTA TTC GAA GAA ACC G-3' and reverse primer 5'-CTA CTC TTC TGA AAA GCG TCG-3'= SCC*mec*IVb forward primer 5'-TCT GGA ATT ACT TCA GCT GC-3' and reverse primer 5'-AAA CAA TAT TGC TCT CCC TC-3'= SCC*mec*IVc forward primer 5'- ACA ATA TTT GTA TTA TCG GAG AGC-3' and reverse primer 5'-TTG GTA TGA GGT ATT GCT GG-3'= SCC*mec*IVd forward primer 5'-CTC AAA ATA CGG ACC CCA ATA CA-3' and reverse primer 5'-TGC TCC AGT AAT TGC TAA AG-3'= SCC*mec*V forward primer 5'-GAA CAT TGT TAC TTA AAT GAG GG-3' and reverse primer 5'-TGA AAG TTG TAC CCT TGA CAC C-3'. The expected amplicon sizes were as follows: SCC*mec*I, 613bp= SCC*mec*II, 398bp= SCC*mec*III, 280bp= SCC*mec*IVa, 776bp= SCC*mec*IVb, 493bp= SCC*mec*IVc, 200bp= SCC*mec*IVd, 881bp= SCC*mec*V, 325bp.

Detection of *lukFS-PV* gene

PCR for detection of *lukFS-PV* gene was done using the *lukFS-PV* primer sequences of 5'-ATCATTAGGTAAAATGTACTGGACATGATCA-3' and 5'-CATCAATGTATTGGATAGCAAAAGC3' respectively. The strains analysed were selected randomly to represent those obtained from all halls of residence and from students of different socio-demographics. In every PCR tube, 4**µl of the** ready to mix 5x FIREPol®Master Mix (Solis Biodyne), 0.4**µl of both forward and reverse primers at a concentration of 10pmol, 1 µl of BSA, 12.2 µl of RNase/DNase free PCR water and 2 µl of sample DNA were added. Amplification reactions were set at an initial denaturation temperature of** 94°C for 5 minutes. Thereafter, the preparation was subjected to 30 cycles at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 60 seconds, final extension at 72°C for 7 minutes and a holding temperature set at 4°C. The expected product size was 433 bp. A control strain was included as positive control in all reactions.

Repetitive sequence-based (Rep)-PCR genotyping

Selection of isolates for Rep-typing was made with a view to represent strains from

participants of all socio-demographic characteristics and to represent strains of different antimicrobial resistance patterns. The Staphrep primer with the following base sequence= 5'-TCGCTCAAAACAACGACACC-3' was used at a concentration of 75pmol per 31.5-µl assay. Amplification reactions were done using the Pure-Taq (Ready-to-go) PCR beads (GE Healthcare), 1.5µl of the Staphrep primer listed in table 1, 25µl of RNase/DNase free PCR water and 5µl of sample DNA. Thermal cycling parameters were set follows: initial denaturation at 95°C for 7 minutes followed by 31 cycles of 94°C for 1 minute, annealing at 40°C for 1 minute, extension at 65°C for 8 minutes and a final extension at 65°C for 15 minutes. A holding temperature was set at 10°C. A control strain was included as a marker. The rep-PCR banding patterns were analysed using GelCompar II (Biomerieux Company).

Visualization of PCR products

PCR products were visualized under a UV trans illuminator on 1.2% agarose gel loaded with ethidium bromide.

Statistical analysis

Statistical analysis was performed using the SPSS version 21 (IBM Corporation). Chi-square and Fisher exact tests were used for analyses of bivariate variables while odds ratios were used for analyses of the association between nasal carriage and demographic factors. The level of significance was set at 95% CI.

Ethical considerations

An approval to conduct this study was sought from the Kenya Medical Research Institute`s Scientific and Ethics Review Unit. Permission to visit, recruit, interview and sample the students was sought from the University's administration. Also, prior to recruitment, a written consent was obtained from students.

RESULTS

General characteristics of study participants

All participants in this study were aged between 18 and 30 years. Characteristics of these participants are captured in table 1 below. Carriage of S. aureus and possible risk factors for colonization among study participants

A total of 231 isolates were recovered. Of these, 78(33.8%) were obtained from the participants noses while 87(37.7%) and 66(28.6%) were obtained from phones and pens respectively. Carriage of S. aureus with respect to the different variables and their specific categories analyzed in this study is provided in table 2 below.

Nasal carriage of S. aureus was significantly higher among females students 47(40.2%) compared to males 31(26.7%) (p=0.02, OR=0.52, CI=0.29-0.93). However, we also found out that colonization of nostrils was more common among male students who participated in sports than females (p=0.03, OR= 3.05, CI= 0.99-9.59). Analysis also revealed that nasal carriage was significantly higher among participants occupying halls 4 (a female`s hostel) and 3 (a male`s hostel) in comparison to other halls (p=0.001, OR= 3.28 CI= 1.58-6.83). We also established that nasal colonization was significantly higher among those who stayed in groups of four (congested conditions) than in those who stated in lesser numbers (p=0.003, OR= 2.36, CI= 1.28-4.39).

Table 1
Descriptive statistics of the sampled university students, 2016

Variable	Categories	Frequency	Percentage
Gender	Males	120	50.6%
	Females	117	49.4%
Age	18-30 years	237	100%
Resident halls	Hall 1	48	20.3%
	Hall 2	39	16.5%
	Hall 3	13	5.5%
	Hall 4	30	12.7%
	Hall 5	9	3.8%
	Hall 6	98	41.4%
Students per room	In Fours	124	52.3%
	In Threes	11	4.6%
	In Twos	102	43%
Last time of hospitalization	Less than 48 hours ago	0	0%
	More than 48 hours ago	237	100%
Sporting activities	Participated	80	33.8%
	Did not participate	157	66.2%
Disinfection of pens and phones	Disinfected	12	5.1%
	Did not disinfect	225	94.9%
Sharing of phones pens	Shared	223	94.1%
	Did not share	14	5.9%
Sharing of personal effects	Shared	57	24.1%
	Did not share	180	75.9%
Cleaning of nostrils	Used handkerchief	214	90.3%
	Used fingers	9	3.8%
	Used other means	8	3.4%
	Never cleaned	6	2.5%
Washing hands	Used medicated soaps	148	62.4%
	Used non-medicated soaps	89	37.6%

*Type of accommodation is the number of students in a hostel room= personal effects represented items like clothes, bed sheets, towels and tooth brush

Table 2
Carriage of *S. aureus* in the sampled student population

Variable	Categories	Number of students tested	Colonization on a single site			Colonization on multiple sites			
			Nostril n (%)	Phone n (%)	Pens n (%)	Nostrils and phone	Nostril and pen	Phone and pen	All sites
Gender	Males	120	31 (26.7%)	38 (31.7%)	30 (25%)	15 (12.5%)	14 (11.7%)	14 (11.7%)	9 (7.5%)
	Females	117	47 (40.2%)	49 (41.8%)	36 (30.8%)	25 (21.4%)	15 (12.8%)	17 (14.5%)	8 (3.4%)
Resident halls	Hall 1	48	15 (31.1%)	16 (33.3%)	17 (35.4%)	6 (12.5%)	4 (8.3%)	6 (12.5%)	1 (2.1%)
	Hall 2	39	15 (38.5%)	20 (51.3%)	10 (25.6%)	10 (25.6%)	6 (15.4%)	7 (17.9%)	4 (10.3%)
	Hall 3	13	7 (53.8%)	5 (38.5%)	3 (23.1%)	2 (15.4%)	1 (7.7%)	2 (15.4%)	1 (7.7%)
	Hall 4	30	17 (56.7%)	12 (40%)	9 (30%)	9 (30%)	4 (13.3%)	4 (13.3%)	3 (10%)
	Hall 5	9	3 (33.3%)	4 (44.4%)	3 (33.3%)	2 (22.2%)	2 (22.2%)	2 (22.2%)	2 (22.2%)
	Hall 6	98	21 (21.4%)	29 (29.6%)	24 (24.5%)	12 (12.2%)	11 (11.2%)	10 (10.2%)	6 (6.1%)
Occupancy	In Fours	124	52 (41.9%)	49 (39.5%)	37 (29.8%)	25 (20.2%)	17 (13.7%)	20 (16.1%)	11 (8.9%)
	In Threes	11	3 (27.3%)	4 (36.4%)	3 (27.3%)	4 (36.4%)	0 (0%)	0 (0%)	0 (0%)
	In Twos	102	23 (22.5%)	32 (31.4%)	26 (25.5%)	12 (11.8%)	12 (11.8%)	11 (10.8%)	6 (5.9%)
Sporting activities	Participated	80	22 (27.5%)	30 (37.5%)	21 (26.3%)	12 (15%)	11 (13.8%)	12 (15%)	8 (10%)
	Did not participate	157	57 (36.3%)	57 (36.3%)	45 (28.7%)	29 (18.5%)	18 (11.5%)	19 (12.1%)	9 (5.7%)
Disinfection of pens and phones	Disinfected	12	4 (33.3%)	4 (33.3%)	3 (25%)	1 (8.3%)	1 (8.3%)	1 (8.3%)	0 (0%)
	Did not disinfect	225	75 (33.3%)	83 (36.9%)	63 (28%)	40 (17.8%)	28 (12.4%)	30 (13.3%)	17 (7.6%)
Sharing of phones/pens	Shared	223	75 (33.6%)	84 (37.7%)	62 (27.8%)	40 (17.9%)	26 (11.7%)	30 (13.5%)	16 (7.2%)
	Did not share	14	4 (28.6%)	3 (21.4%)	4 (28.6%)	1 (7.1%)	3 (21.4%)	1 (7.1%)	1 (7.1%)

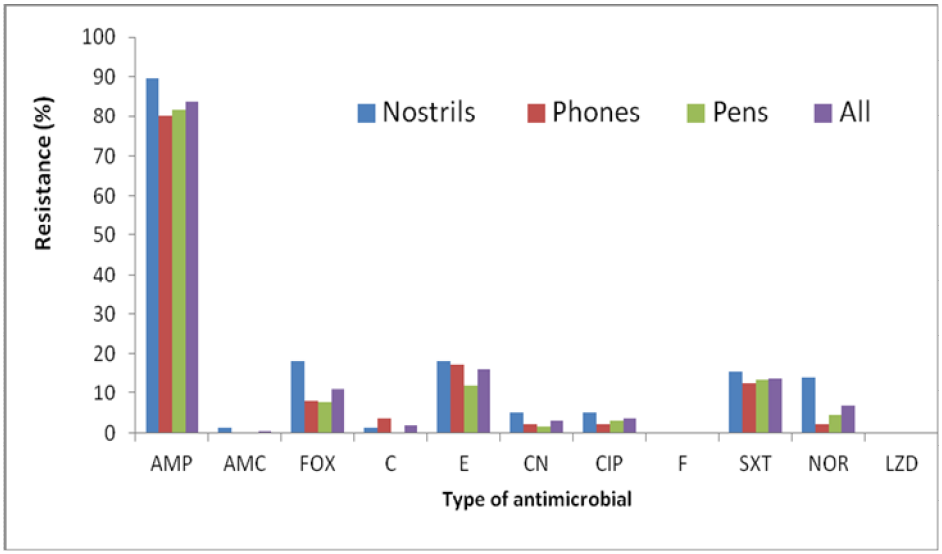
Sharing of personal effects	Shared	57	20 (35.1%)	23 (40.4%)	14 (24.6%)	14 (24.6%)	5 (8.8%)	7 (12.3%)	4 (7%)
	Did not share	180	59 (32.8%)	64 (35.6%)	52 (31.7%)	27 (15%)	24 (13.3%)	24 (13.3%)	13 (7.2%)
Cleaning of nostrils	Used handkerchief	220	72 (32.7%)	80 (36.4%)	63 (28.6%)	38 (17.3%)	29 (13.2%)	28 (12.7%)	17 (7.7%)
	Used fingers	9	3 (33.3%)	3 (33.3%)	1 (11.1%)	2 (22.2%)	0 (0%)	1 (11.1%)	0 (0%)
	Used other means	8	3 (37.5%)	3 (37.5%)	2 (25%)	1 (12.5%)	0 (0%)	2 (25%)	0 (0%)
Washing hands	Used medicated soaps	148	48 (32.4%)	59 (39.9%)	43 (29.1%)	29 (19.6%)	19 (12.8%)	22 (14.9%)	13 (8.8%)
	Used non-medicated soaps	89	31 (34.8%)	28 (31.5%)	23 (25.8%)	12 (13.5%)	10 (11.2%)	9 (10.1%)	4 (4.5%)

*Type of accommodation is the number of students in a hostel room= personal effects represent items like clothes, bed sheets, towels and tooth brush

Antimicrobial resistance profiles of isolates recovered from the student population

The 231 isolates were tested against 11 antimicrobials. In general, we found low resistances to erythromycin (16%), trimethoprim-sulfamethoxazole (13.9%), cefoxitin, (11.3%), norfloxacin (6.9%), gentamicin (3%), ciprofloxacin (3.5%), chloramphenicol (1.8%) and amoxicillin-clavulanic acid (0.4%). However, higher resistances of above 80% were recorded for ampicillin (84%). Most isolates showed high resistance to ampicillin regardless of the source. Amoxicillin-clavulanic acid-resistance was recorded in a single isolate recovered from the nostrils. With regard to the sites of isolation, the prevalence of cefoxitin-resistant isolates also refereed henceforth as MRSA strains was 18% for isolates recovered from nostrils compared to phones (8.1%) and pens (7.6%). In addition, the proportion of isolates from nose that were resistant to gentamicin and ciprofloxacin (both 5.1%), trimethoprim-sulfamethoxazole (15.4%) and norfloxacin (14.1%) was slightly higher than the proportion of isolates recovered from fomites. A high proportion of isolates from nostrils were also resistant to erythromycin (17.2%) compared to from phones (17.2%) and pens (12.1%). Chloramphenicol resistance was high among isolates recovered from phones (3.5%) compared to nostrils (1.3%) while no resistance was recorded for isolates recovered from the pens (Figure 1).

Figure 1
Antimicrobial resistance patterns of isolates from nostrils and fomites

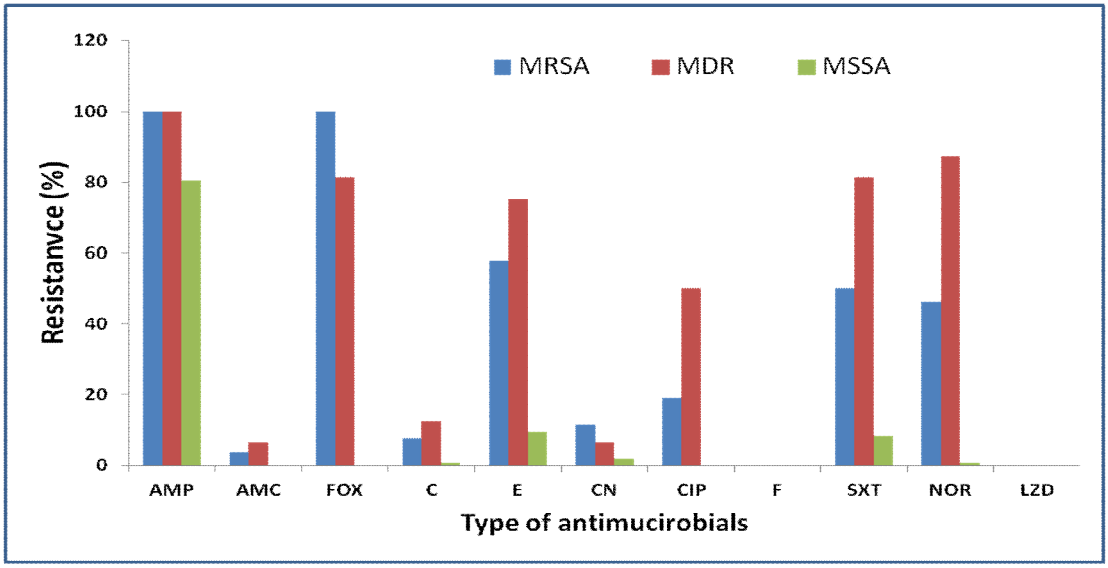


*AMP, ampicillin= AMC, amoxicillin-clavulanic acid= FOX, cefoxitin= C, chloramphenicol= E, erythromycin= CN, gentamicin= CIP, ciprofloxacin= F, nitrofurantoin= SXT, trimethoprim-sulfamethoxazole= NOR, norfloxacin= LZD, linezolid= All, all isolates from nostrils, phones and pens combined.

Resistance phenotypes associated with MRSA, MDR and MSSA strains

Our study revealed that all the MRSA and MDR strains were resistant to ampicillin while 80.5% of the MSSA were susceptible to this antimicrobial. In addition, high resistances (above 40%) were observed for erythromycin, norfloxacin and trimethoprim-sulfamethoxazole among the MRSAs compared to lower resistances of below (10%) for the same antimicrobials among the MSSA. Low resistance levels of less than 20% were recorded for amoxicillin-clavulanic acid, chloramphenicol and gentamicin among the MDRA and MDRS strains. Also, a majority of the MRSAs (57.7%) and MDR strains (75%) showed resistance to erythromycin (Figure 2). We also established that MRSA strains isolates were more likely to display multi-drug resistance compared MSSA strains (P=0.001).

Figure 2
Antimicrobial resistance patters of *S. aureus* isolates obtained from the study population



*MRSA, methicillin-resistant *S. aureus,* MDR, multi-drug resistant, MSSA, methicillin-susceptible *S. aureus*= AMP, ampicillin= AMC, amoxicillin-clavulanic acid= FOX, cefoxitin= C, chloramphenicol= E, erythromycin= CN, gentamicin= CIP, ciprofloxacin= F, nitrofurantoin= SXT, trimethoprim-sulfamethoxazole= NOR, norfloxacin= LZD, linezolid

Prevalence and possible epidemiologic factors associated with carriage of MRSA and MDR strains among study participants

Out of the 231 isolates, 26 (11.3%) were MRSA while 16 (6.93%) were MDRs. We also found that 50% of the MRSAs were also MDRs. The carriage of MRSA and MDRs among the study population is provided in table 3 below. Our study showed that the gender of participants was not significantly associated with the risk of colonization by MRSAs or MDRs on nostrils or fomites (p>0.05). Isolates recovered from halls two, four and five were more likely to be MRSAs as opposed to those recovered from halls one, three and six (P= 0.03, OR= 2.46, CI= 0.98-6.25). We also

established that those who did not disinfect their phones were more likely to carry an MRSA strain in the nose or on their fomites (P=0.02, OR=6.03, CI= 1.30-26.86).

Table 3
Proportions of MDRs and MRSA recovered from study participants

Variables	Categories	Total number of *S. aureus* isolates (n)	Total number of MDRs, n (%)	Number of MRSA strains, n (%)
Gender	Males	99	4 (4%)	9 (9%)
	Females	132	12 (9.1%)	17 (12.9%)
Hall of residence	Hall one	49	3 (6.1%)	4 (8.2%)
	Hall two	45	4 (8.9%)	5 (11.1%)
	Hall three	15	0 (0%)	1 (6.7%)
	Hall four	38	5 (13.2%)	8 (21.1%)
	Hal five	10	1 (10%)	2 (20%)
	Hall six	74	3 (4.1%)	6 (8.2%)
Type of accommodation	Twos	80	3 (3.8%)	7 (8.8%)
	Threes	12	2 (16.7%)	3 (25%)
	Fours	139	11 (7.9%)	16 (11.5%)
Sporting activities	Participated	74	6 (8.1%)	9 (12.2%)
	Did not participate	157	10 (6.4%)	17 (10.8%)
Disinfection of pens and phones	Disinfected	10	1 (10%)	4 (40%)
	Did not disinfect	221	15 (6.8%)	22 (10%)
Sharing phones and pens	Shared	220	15 (6.8%)	25 (11.4%)
	Did not share	11	1 (9.1%)	1 (9.1%)
Sharing personal effects	Shared	57	2 (3.5%)	7 (12.3%)
	Did not share	174	14 (8.0%)	19 (10.9%)
Cleaning of nostrils	Used a handkerchief	215	14 (6.5%)	24 (11.2%)
	Used fingers	6	0 (0%)	0 (0%)
	Cleaned by other means	10	1 (10%)	2 (20%)
Bathing or washing clothes	Used medicated soaps	149	13 (8.7%)	21 (14.1%)
	Used non-medicated soaps	82	3 (3.7%)	5(6.1%)

**Type of accommodation is the number of students in a hostel room= Personal effects represent items like clothes, bed sheets, towels and tooth brush, MRSA, methicillin-resistant S. aureus, MDR, multi-drug resistant, MSSA, methicillin-susceptible S. aureus*

Prevalence of *spa, mecA*, SCC*mec* elements and *lukFS-PV* genes among the *S. aureus* isolates

All the isolates carried the Spa genes indicating they were *Staphylococcus* strains. As expected, majority (65.4%) of the MRSA harboured the *mecA* gene and 52.9% of these were also MDRs. The most common *mec*-cassette was SCC*mec*V (6.9%) followed by SCC*mec*II (6.5%). SCC*mec*III was present in only 1.3% of the isolates. None of the SCC*mec*IV types was found among the isolates. SCCmecII was highly prevalent among the MDRs (68.8%) than among MRSA strains (53.9%). Similarly, SCC*mec*V was highly prevalent among the MDRs (62.5%) and MRSA (61.5%) compared to MSSA strains, which did not harbour this element. SCC*mec*III was present in a small proportion (11%) of the MRSA strains (Table 4). Carriage of the *lukFS-PV* gene within our isolates was only at 31.5% and only 26.9% of the MRSA and MSSA strains (table 5), and there was no statistical association between the two strains with regard to carriage of the gene (p=0.487).

Table 4
Proportion of *S. aureus* isolates harbouring *spa, mecA*, SCC*mec* elements and *lukFS-PV* genes among the *S. aureus* isolates

Type/source of isolate	Type of gene (N=231)				
	MecA	SCC*mec*I	SCC*mec*II	SCC*mec*III	SCC*mec*V
Overall	17 (7.4%)	7 (3.0%)	15 (6.5%)	3 (1.3%)	16 (6.9%)
MRSA	17 (65.4%)	4(15.4%)	14 (53.9%)	3 (11.5%)	16 (61.5%)
MSSA	0 (0%)	2 (1.0%)	0	0	0
MDR	9 (56.3%)	1 (6.3%)	11 (68.8%)	0 (0%)	10 (62.5%)
Nostril	8 (10.3%)	3 (3.9%)	10 (12.8%)	2 (2.6%)	10 (12.8%)
Fomites	9 (5.9%)	4 (2.6%)	5 (3.3%)	1 (0.7%)	6 (3.9%)

**The prevalences for SCCmec Iva, SCC mec IVb, SCC mec IVc and SCC mec IVd were 0%, and therefore, were not included in the table= MRSA, methicillin-resistant S. aureus= MDR, multi-drug resistant= MSSA, methicillin-susceptible S. aureus= Overall, all the isolates*

Table 5
Proportion of *S. aureus* isolates harbouring *lukFS-PV* genes *S. aureus* isolates

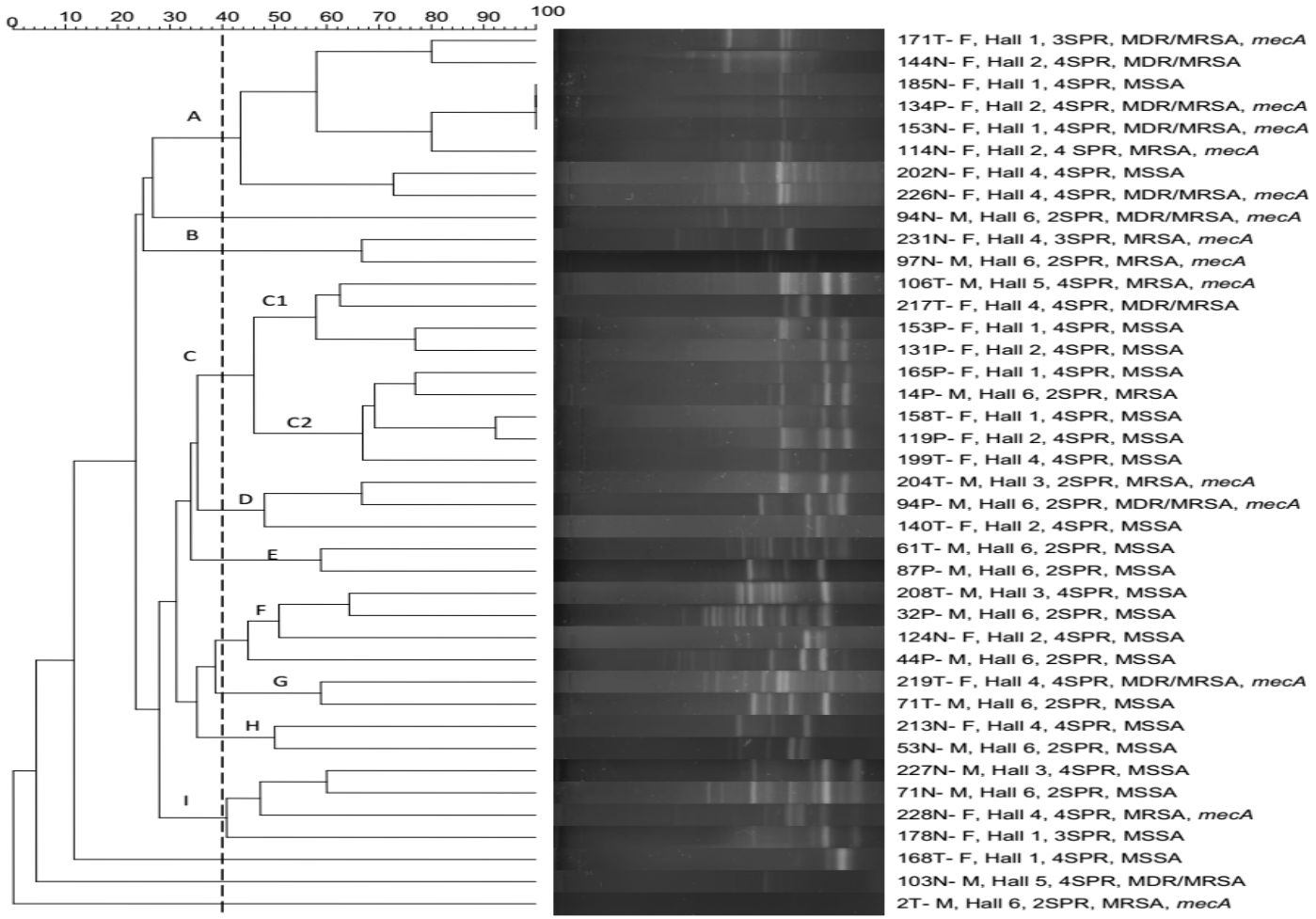
Type/source of isolate	Number of isolates tested	***lukFS-PV*** gene, n (%)
Overall	54	17 (31.5%)
MRSA	26	7 (26.9%)
MSSA	28	10 (35.7%)
MDR	16	4 (25%)
Nostril	22	7 (31.8%)
Fomites	32	10 (31.3%)

**MRSA, methicillin-resistant S. aureus= MDR, multi-drug resistant= MSSA, methicillin-susceptible S. aureus= Overall, all isolates that were selected to screen for the presence of the* lukFS-PV *gene*

Genetic relationships of *S. aureus* isolates recovered from the student population

A total of 40 isolates with different resistance phenotypes obtained from participants with different clinical and socio-demographic backgrounds were selected and analysed for genetic relatedness using the Repetitive element Sequence-Based PCR. At a 40% level of similarity, the 40 isolates fit into 10 different clusters identified as Cluster A to I (Figure 3). Cluster A isolates were all from female students. About 62.5% of the isolates in this cluster were MRSA that were also MDR. All the Isolates in cluster C were recovered from fomites. These isolates fit into two sub-clusters (C1 and C2). All isolates in sub-cluster C1 were recovered from participants who stayed in fours. All isolates in sub-cluster C2 (55.6%) were recovered from students who did not take part in sports and were also negative for *mecA* gene, although one of them was an MRSA. At least 90.9% of isolates in clusters D to G were recovered from fomites used by males mostly (72.7%) than females (27.3%). These isolates were found to circulate within halls two, three and six. All the isolates in clusters H and I were recovered from nostrils of both males and females in equal proportions. These isolates were also MSSA strains and were circulating with halls one, two, three, four and six.

Figure 3
Rep-PCR-generated dendogram for selected *S. aureus* isolates



*F, female= M, male= SPR, number of students per room= MDR/MRSA, Multi-drug resistant/methicillin-resistant *S. aureus*= MSSA, methicillin-susceptible *S. aureus.*

DISCUSSION

The approximate rate of *S. aureus* colonization among students in institutions of higher learning has been varied with prevalences below 25% being reported for nasal colonization in China and Nepal (12,13). In this study, we found prevalences above 25% for both nasal and fomites colonization. We found no reports highlighting the prevalences of colonization of fomites such as phones and pens, specifically those used by students in university settings in Kenya.

Consistent with the findings by Hogan et al. (14), the female gender was a risk factor for nasal colonization. Significantly high carriage of *S. aureus* and MRSA on nostrils and phones were found in several halls where students stayed in congested rooms of at least 4 occupants per room numbers. Possibly, this type of accommodation also facilitated sharing items such as phones, pens and other personal effects. Turabelidze et al. (15) reported that sharing such objects is a risk factor for the colonization by *S. aureus* and related MRSA strains. Kejela et al. (3) also reported congestion or overcrowding to be a possible risk factor for colonization by MRSA strains among student populations.

We found high resistance to ampicillin among the isolates in this study. Okamo et al. (16) and Kejela et al. (3) also reported high ampicillin resistance among MRSA isolates recovered from a healthy student populations. All the isolates were sensitive to nitrofurantoin and linezolid, a finding that confirmed results reported by Kitti et al. (17) and Eko et al. (18). These findings could possibly suggest that nitrofurantoin and linezolid may be used effectively in the management of *S. aureus* related infections, a recommendation that was given by Watkins et al. (19). Resistances to chloramphenicol, erythromycin, gentamicin, ciprofloxacin, norfloxacin trimethoprim-sulfamethoxazole have been variable across different studies (14,18,20), an observation that prompts the necessity of continued screening of *S. aureus* isolates to determine the efficacy of the antimicrobials prior to their use in managing *S. aureus*-related infections.

Our results established the existence of a relatively low prevalence of MRSA among a healthy population of university students but other studies have reported higher MRSA prevalence (18.7%) among student populations in Jeddah, Saudi Arabia (21). Other studies in Tanzania (16) and (17) have reported very low prevalences of 0.3% and 1% respectively among student populations. Hence, considering the case of this study where significantly high chances of MRSA colonization were reported in particular residence halls, it could be possible that other students visiting these residence halls would be increasing their chances of MRSA colonization.

Results from this study showed that SCC*mec*II and V were predominant among the isolates from the healthy student population. In Kenya, the SCC*mec*II, III, IV have been found to be most predominant is a pool of isolates recovered from health care settings in different studies (11,22,23). We did not find studies that reported the prevalence of these elements among isolates recovered from healthy populations. The predominance of SCC*mec*II could be attributed to their small size, and therefore, can be transmitted with ease within community settings (23). Due to scarcity of information on the prevalence of SCCmec elements within community settings, more studies need to be done in order to ascertain why certain elements are predominant within specific populations in Kenya. We did not find a statistically

significant association between carriage of the *lukFS-PV* and MRSA or MSSA strains. However, other studies have reported statistically significant increase in the prevalence of *pvl*-positive MRSA strains from clinical settings in regions such as Ireland and Germany (24–26). A study by Schaumburg et al. (27) reported a high prevalence (55.9%) of isolates from a health population carrying the *lukFS-PV* gene, but the reason and impact of this was unclear. We found a prevalence of 31.5%, which suggested a likelihood of existence of virulent strains among healthy populations.

Analysis of genetic relations of the isolates indicated a likelihood of strain sharing among students. This would be expected since the isolates were recovered from students who stayed in close proximities, and shared their personal items.

CONCLUSION

This study offers insights into the prevalence of carriage of *S. aureus* and associated MRSA strains in the nasal cavities, phones and pens of students in a university setting in Central Kenya. Among the factors found to be associated with increased transmission rates of *S. aureus*, was congestion in some hostel rooms (staying in fours). Based on this information, we find it important to allow students to stay in fewer numbers. Since we found no studies, which reported rates of colonization in students residing in different hostels within a single institution of higher learning, similar studies could help identify the public health significance of reducing the number of students sharing a hostel room to less than four. There should also be comprehensive surveillance studies on MRSA and control measures to help in curbing the spread of MRSA strains.

COMPETING INTERESTS

The authors declare that they have no competing interests

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REFERENCES

1. DeLeo FR, Otto M, Kreiswirth BNB, Chambers HFH. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* [Internet]. 2010;375(9725):1557–68. Available from: http://www.sciencedirect.com/science/article/pii/S0140673609619991%5Cnhttp://dx.doi.org/10.1016/S0140-6736(09)61999-1
2. Brown AF, Leech JM, Rogers TR, McLoughlin RM. *Staphylococcus aureus* colonization: Modulation of host immune response and impact on human vaccine design. *Front Immunol.* 2014;4(JAN):1–20.
3. Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) among primary school children and prisoners in Jimma Town, Southwest Ethiopia. *Ann Clin Microbiol Antimicrob* [Internet]. 2013;12(1):11. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L52619231%5Cnhttp://www.ann-clinmicrob.com/content/12/1/11%5Cnhttp://dx.doi.org/10.1186/1476-0711-12-11%5Cnhttp://sfx.hul.harvard.edu/sfx_local?sid=EMBASE&issn=14760711&id=doi:10.118
4. Walia SS, Manchanda A, Narang RS, Anup N, Singh B, Kahlon SS. Cellular telephone as

reservoir of bacterial contamination: Myth or fact. *J Clin Diagnostic Res.* 2014;8(1):50–3.

5. Arulmozhi V, Janagond AB, Savitha S, Kalyani J, Sumathi G. Cell phones of healthcare providers as fomites harbouring potential pathogenic microogranisms. *Int J Curr Res Rev* [Internet]. 2014;6(19):1–4. Available from: http://www.scopemed.org/fulltextpdf.php?mno=170604
6. Otto M. Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. *Clin Epidemiol* [Internet]. 2013;5:205. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3707418&tool=pmcentrez&rendertype=abstract
7. CDC. Antibiotic Resistance Threats. *Cdc.* 2013;22–50.
8. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. *P & T: A peer-reviewed J Formul Manag* [Internet]. 2015;40(4):277–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25859123%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4378521%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25859123%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4378521
9. Mozzillo KL, Ortiz N, Miller LG. Patients with Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infection – 21st Century Lepers. *NIH Public Access*. 2010;75(2):132–4.
10. Braga EDV, Aguiar-Alves F, de Freitas M de FN, de e Silva MO, Correa TV, Snyder RE, et al. High prevalence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* colonization among healthy children attending public daycare centers in informal settlements in a large urban center in Brazil. *BMC Infect* Dis [Internet]. 2014;14:538. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4287590&tool=pmcentrez&rendertype=abstract
11. Maina EK, Kiiyukia C, Wamae CN, Waiyaki PG, Kariuki S. Characterization of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in patients in Nairobi, Kenya. *Int J Infect Dis* [Internet]. 2013;17(2):e115–9. Available from: http://dx.doi.org/10.1016/j.ijid.2012.09.006
12. Du J, Chen C, Ding B, Tu J, Qin Z, Parsons C, et al. Molecular characterization and antimicrobial susceptibility of nasal *Staphylococcus aureus* isolates from a chinese medical college campus. *PLoS One.* 2011;6(11).
13. Ansari S, Gautam R, Shrestha S, Ansari SR, Subedi SN, Chhetri MR. Risk factors assessment for nasal colonization of *Staphylococcus aureus* and its methicillin resistant strains among pre-clinical medical students of Nepal. *BMC Res Notes* [Internet]. 2016;9(1):214. Available from: http://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-016-2021-7
14. Hogan B, Rakotozandrindrainy R, Al-emran H, Dekker D, Hahn A, Jaeger A, et al. Prevalence of nasal colonisation by resistant *Staphylococcus aureus* among healthcare workers and students in Madagascar. *BMC Infect Dis* [Internet]. 2016;1–9. Available from: http://dx.doi.org/10.1186/s12879-016-1733-6
15. Turabelidze G, Lin M, Wolkoff B, Dodson D, Gladbach S, Zhu BP. Personal hygiene and methicillinresistant *Staphylococcus aureus* infection. *Emerg Infect Dis.* 2006;12(3):422–7.
16. Okamo B, Moremi N, Seni J, Mirambo MM, Kidenya BR, Mshana SE. Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* nasal carriage among pre-clinical and clinical medical students in a Tanzanian University. *BMC Res Notes* [Internet]. 2016;9(1):47. Available from: http://www.biomedcentral.com/1756-0500/9/47
17. Kitti T, Boonyonying K, Sitthisak S. Prevalence of methicillin-resistant *Staphylococcus aureus* among university students in Thailand. *Southeast Asian J Trop Med Public Health.* 2011;42(6):1498–504.
18. Eko KE, Forshey BM, Carrel M, Schweizer ML, Perencevich EN, Smith TC. Molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization and infection isolates in a Veterans Affairs hospital. *AntimicrobResistInfectControl*. 2015;4(2047–2994 (Electronic)):10.

19. Watkins RR, Lemonovich TL, File TM. An evidence-based review of linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA): Place in therapy. *Core Evid.* 2012;7:131–43.
20. Ayepola OO, Olasupo NA, Egwari LO, Becker K, Schaumburg F. Molecular characterization and antimicrobial susceptibility of *Staphylococcus aureus* isolates from clinical infection and asymptomatic carriers in Southwest Nigeria. *PLoS One.* 2015;10(9):4–11.
21. Zakai SA. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal colonization among medical students in Jeddah, Saudi Arabia. *Saudi Med J.* 2015;36(7):807–12.
22. Aiken AM, Mutuku IM, Sabat AJ, Akkerboom V, Mwangi J, Scott JAG, et al. Carriage of *Staphylococcus aureus* in Thika Level 5 Hospital, Kenya: a cross-sectional study. *Antimicrob Resist Infect Control* [Internet]. 2014;3:22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4107749&tool=pmcentrez&rendertype=abstract
23. Omuse G, Van Zyl KN, Hoek K, Abdulgader S, Kariuki S, Whitelaw A, et al. Molecular characterization of *Staphylococcus aureus* isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study. *Ann Clin Microbiol Antimicrob* [Internet]. 2016;15(1):51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27647271%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5029008
24. Melles, Damian C; van Leeuwen, Willem B; Boelens, Helene AM;Peeters, Justine K;Verbrugh H a; VB. Panton-Valentine Leukocidin Genes in *Staphylococcus* Small Anellovirus in Hepatitis C Patients and Healthy Controls. *Emerg Infect Dis.* 2006;12(7):1174–5.
25. Berktold M, Grif K, Mäser M, Witte W, Würzner R, Orth-Höller D. Genetic characterization of Panton-Valentine leukocidin-producing methicillin-resistant *Staphylococcus aureus* in Western Austria. *Wien Klin Wochenschr.* 2012;124(19–20):709–15.
26. Shore AC, Tecklenborg SC, Brennan GI, Ehricht R, Monecke S, Coleman DC. Panton-Valentine leukocidin-positive *Staphylococcus aureus* in Ireland from 2002 to 2011: 21 clones, frequent importation of clones, temporal shifts of predominant methicillin-resistant S. aureus clones, and increasing multiresistance. *J Clin Microbiol.* 2014;52(3):859–70.
27. Schaumburg F, Kock R, Friedrich AW, Soulanoudjingar S, Ngoa UA, von Eiff C, et al. Population structure of *Staphylococcus aureus* from remote African Babongo Pygmies. *PLoS Negl Trop Dis.* 2011;5(5).