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Antimicrobial resistance profile of *Staphylococcus aureus* isolated from patients, healthcare workers, and the environment in a tertiary hospital in Addis Ababa, Ethiopia

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Abstract

Staphylococcus aureus infection and colonization in patients may be transmitted to healthcare providers and the environment and subsequently cause healthcare-associated infections in other patients. Pathogenic *S. aureus* strains produce virulence factors, such as Panton-Valentine Leukocidin (PVL), that contribute to the severity of infections and aid in their spread. The emergence of antimicrobial resistance (AMR) is additional concern with respect to *S. aureus* infection. In this study, the virulence genes and antibiotic resistance profiles of *S. aureus* were characterized from patients' clinical isolates, healthcare workers' (HCWs') nasal colonization screenings, and the environment at a tertiary healthcare hospital in Addis Ababa, Ethiopia. A total of 365 samples were collected from September 2021 to September 2022: 73 patients' clinical specimens, 202 colonization screenings from HCWs, and 90 hospital environment's swabs. Fifty-one (25.2%) HCW and 10/90 (11.1%) environment *S. aureus* isolates were identified. Among the 134 isolates, 10 (7.5%) were methicillin-resistant *S. aureus* (MRSA). Three (4.1%), five (9.8%), and two (20.0%) of the MRSA isolates were identified from the patients, HCWs, and the environment, respectively. Overall, 118 (88.1%) were ampicillin and penicillin resistant; 70 (52.2%) were trimethoprim sulfamethoxazole resistant; and 28 (20.9%) were erythromycin resistant. *S. aureus* isolates from patients were more resistant to antibiotics than isolates from HCWs or the hospital environment ($p<0.05$). A total of 92/134 (68.6%) isolates possessed the *lukf*-PV gene, which was identified in 62 (85.0%), 26 (51.0%), and 4 (40.0%) of the patient, HCWs, and the environment, respectively. The proportion of *lukf*-PV gene containing *S. aureus* isolated from patient samples was statistically significant. Four (40.0%) of the MRSA isolates also had the *lukf*-PV gene. The identification of highly AMR and virulence factors from patients, HCWs

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and the environment is concerning. Further studies are needed to identify potential transmission links and improve infection prevention and control.

Introduction

Staphylococcus aureus is a widespread organism that is also found as a normal flora of human body. Approximately 20–30% of the human populations are reported to be colonized with persistent nasal carriage of *S aureus*, while 60% are intermittent carriers, and another 20% are non-carriers [1, 2]. *S. aureus* colonization of patients has been connected to subsequent infection [3], and healthcare workers (HCWs) have been identified as potential reservoirs and possible source for the cross-transmission of pathogens associated with healthcare-associated infections (HAIs) [4, 5].

S. aureus infections can affect any part of the body from minor skin infections to serious, life-threatening pneumonia and bacteremia [6, 7]. The production of virulence factors by pathogenic strains, such as expression of a cell-surface protein that binds and inactivates antibodies, as well as the production of powerful protein toxins like staphylococcal enterotoxin and Panton-Valentine Leukocidin (PVL), can aid in the spread of infections [8] PVL is a pore-forming toxin largely responsible for skin and soft tissue infection. There is a significant variation in the prevalence of PVL among methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) infections worldwide. The prevalence of PVL toxin has been reported to be increasing in Africa [9, 10].

Emergence of antimicrobial resistance (AMR) in healthcare facility is a growing concern due to increasing prevalence of multidrug resistant organisms (MDROs) and limited availability of effective antimicrobial therapeutics for treatment. In Ethiopia, the majority of studies on *S. aureus* AMR patterns rely on culture-based pathogen identification and drug susceptibility testing. However, there is a limited understanding of the molecular resistance and virulence gene profiles of *S. aureus* in Ethiopia [14]. This study aimed to characterize the antibiotic resistance and virulence gene profiles of *S. aureus* isolates obtained from patient clinical samples, healthcare worker colonization screenings, and environmental sampling at a tertiary healthcare hospital in Addis Ababa, Ethiopia.

Material and methods

Study design, sampling procedures and data collection

This cross-sectional study was conducted at St Paul's Hospital Millennium Medical College (SPHMMC) in Addis Ababa, Ethiopia. Patients' clinical specimen, healthcare workers' nasal swab and hospital environment swabs were the source of samples utilized in this study.

Patients: All clinical specimens submitted for routine culture that was positive for *S. aureus* at the SPHMCC from September 2021 to September 2022 were included in the study. The isolates were kept at -80 freezer using 20% glycerol and tryptic soya broth in a cryo-tube for further testing. Patient demographics, diagnosis, specimen type and hospital ward location at the time of specimen submission were collected from hospital medical records. The medical record of the patients was accessed from October 3–7, 2022 and isolates were subculture for testing at October 10, 2022.

Healthcare workers (HCWs): Nasal swabs were collected from volunteer HCWs (doctors, nurses, laboratory personnel, and environmental cleaners) from September 12, 2021 to August 30, 2022. HCWs were recruited from target hospital units (Emergency department (ED),

gynecological (GYN), medical ward (MED), pediatric ward (PEDs), surgical unit (SURG), and laboratory (LAB). HCW sample size was calculated based on a prior colonization study prevalence [15]. Using single population formula, a sample size of 170 was estimated, plus an additional 20% of the sample size, for a total of 202 HCWs. Consent was obtained prior to specimen collection. The HCWs, socio-demographic information including age, sex, years of work experience, and hospital unit location was collected using a short questionnaire. Nasal swab samples were collected from the HCW. The swab was inserted approximately 2 cm into one nostril and rotated against the anterior nasal mucosa for 3 seconds. The same swab was inserted into the other nostril and rotated for 3 seconds. Upon completion, the swab was placed into the Amies transport media with charcoal. The samples were transported to SPHMMC microbiology laboratory. The samples were cultured in mannitol salt agar plate.

Hospital environmental sampling: Environmental swab samples were collected from predetermined the hospital's environment high touch or high contact surfaces and classified into three categories: Patient areas, staff areas (limited to hospital staff), and general public areas. Environmental swab specimens collected from patient areas included patient rooms (intravenous [IV] pole, bed railing, bedside table, and shelf), procedure rooms (table, medical chart, medical chart shelf), and medical supplies (oxygen, mechanical ventilator). Samples collected in staff area included nurse station (medication tray, table), documentation (file tray, medical chart), office supplies (Table, chair and computer), laboratory (chair and bench) and medical supplies and equipment (medication shelf, ultrasound). Samples were also collected from public areas: reception (shelf, hand wash sink); waiting area (chair handle and chair); corridor (elevator buttons and stair railings). Daily cleaning of the environment takes place in the morning, thus all 90 of the environmental samples were all collected after 2:00 pm. Samples were collected using two pre-moisten with sterile saline water swabs and Amies transport media were used to transport the sample to the laboratory [16].

Identification of *S. aureus*

Preliminary *S. aureus* identification was performed at SPHMMC microbiology laboratory using pre-enrichment, non-selective and selective media as previously described [16]. In summary, swabs from HCWs and the environment were placed in 2 mL of tryptone soy broth with NaCl and incubated aerobically at 35˚C for 24 hours, after which 50-μL aliquots of the broth were inoculated on to mannitol salt agar plates. Colonies were selected based on colony morphology and were then sub-cultured on to tryptic soy agar (TSA). Positive catalase and coagulase reactions were considered presumptive positive for *S. aureus*.

Confirmation of MSSA, MRSA and antimicrobial susceptibility

Isolate confirmation and antimicrobial susceptibility testing (AST) were performed for all presumptive isolates at EPHI Clinical Bacteriology and Mycology Laboratory using BD Phoenix M50 system (BD Diagnostic Systems, Franklin Lakes, NJ, USA) version 6.8.1 PMIC/ID-111 panel following manufacturer's procedures [17]. In addition, isolates were determined as either MSSA or MRSA based on the resistance to cefoxitin as determined by minimum inhibitory concentration (MIC> = 8). The isolates were stored at -80˚C in tryptic soy broth (TSB) containing 20% glycerol for further molecular testing.

Nucleic acid isolation and amplification of 16S rRNA, *mecA*, *lukF-PV* and *spa* genes

Genomic DNA was extracted using a commercially available QIAamp DNA Mini kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol [18]. DNA extraction and

polymerase chain reaction (PCR) tests were performed at EPHI. The extract was stored at -20˚C until further analysis. For PCR amplification and analysis of 16S rRNA, *mecA*, *lukF-PV* and *spa* genes, the QIAGEN Microbial DNA qPCR Multi-Assay Kit (QIAGEN, Hilden, Germany) was used according to the manufacturer's procedure [19].

Statistical analysis

STATA version 16.2 (Stata Corp LLC, College Station, TX, USA) was used for data analysis. Descriptive statistics were generated to summarize cefoxitin screening results and *S. aureus* prevalence by sampling site. Logistic regression models were fitted to calculated odds ratio and determine the association between antibiotic resistance and sample source; *lukF-PV* carriage and antimicrobial resistance; MRSA status and independent factors such as age, gender, departments and HCW's years of work experience.

Ethical clearance

The study protocol was approved by the EPHI Institutional Review Board (IRB, EPHI-IRB-029-2017) and SPHHMC IRB (PM23/352). Written consent was obtained from healthcare providers for colonization screening. Retrospective patient data and archived isolates were used; Anonymous analysis was conducted; authors did not have access to information that could identify individual participants, and the ethics committee waived patient consent.

Results

Socio-demographic characteristics of study participants

Patients. Out of 73 patients with *S. aureus* positive culture, 44 (60.3%) were female. The female patients had a mean age of 24.1 years and a median age of 26 years. In contrast, the male patients had a mean age of 16.7 years and a median age of 7 years. For females, the age group of 26–39 made up the majority of participants, whereas for males, it was one year and younger (Table 1).

Health care workers

Out of the 202 HCWs who enrolled and consented to participate in the study, 103 (51.0%) were males. Although the age of HCW range from 21 to 49 years, the majority (65.8%) of them were in the 21–29 age group, with a mean age of 29 and a median age of 28 (Table 2).

Table 1. Distribution of patient (N = 73) age-group, gender, and location in St. Paul's Hospital Millennium Medical College (SPHMMC) in Addis Ababa, Ethiopia.

	Age n (%)	Sex n (%)		Departments n (%)					
Years		**Female**	**Male**	**ED**	**GYN**	**MED**	**OPD**	**PED**	**SURG**
< = 1	20 (27.4)	8 (18.2)	12 (41.4)	0	NA	1 (8.3)	1 (10.0)	18 (72.0)	0
2–14	8 (10.9)	1 (2.3)	7 (24.2)	0	NA	1 (8.3)	0	7 (28.0)	0
15–25	16 (21.9)	13 (29.6)	3 (10.3)	4 (44.4)	1 (11.1)	3 (30.0)	4 (44.4)	NA	4 (36.3)
26–39	20 (27.4)	17 (38.6)	3 (10.3)	3 (33.3)	7 (77.8)	3 (30.0)	3 (44.4)	NA	4 (36.3)
≥ 40	9 (12.3)	5 (11.4)	4 (13.8)	2 (22.2)	1 (11.1)	2 (20.0)	1 (11.1)	NA	3 (27.3)
Total	**73 (100)**	**44 (60.3)**	**29 (34.2)**	**9 (12.3)**	**9(12.3)**	**10 (13.7)**	**9 (12.3)**	**25 (34.3)**	**11 (15.7)**

Abbreviations: ED, Emergency Department; GYN, Gynecology ward; MED, Medicine ward; OPD, outpatient unit; PED, Pediatric ward; SURG, Surgical ward; NA, not applicable.

Notes: 95% Confidence Interval were used.

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Table 2. Distribution of health care workers age-group (N = 202), gender, and location in St. Paul's Hospital Millennium Medical College (SPHMMC) in Addis Ababa, Ethiopia.

Sex		Department	
Female	99 (49.0)	**Emergency**	21 (10.40)
Male	103 (51.0)	**Gynecology**	13 (6.44)
Age group		**Laboratory**	18 (8.91)
21–29	133 (65.8)	**Medicine**	53 (26.24)
30–39	57 (28.2)	**Pediatric**	42 (20.79)
40–49	12 (6.0)	**Surgical**	55 (27.23)

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Identification of MSSA and MRSA from patients, HCWs and environment

Overall, a total of 124 MSSA and 10 MRSA isolates were identified. Out of the 124 MSSA, 70 isolates were from patients' clinical specimen, 46 from HCW nasal colonization, and 8 from hospital environmental source (Fig 1). Of the 10 MRSA isolates, 3 were from patient's clinical specimen, 5 from HCW nasal colonization, and 2 from hospital environmental source. There was no significant difference in MRSA prevalence among different sample sources.

Of the total of 73 patient isolates, 3 (4.1%) were MRSA. The distribution of *S. aureus* isolation from different departments varies with higher percentage in the pediatric ward and the lowest in the outpatient department (Table 1).

Among the total of 202 HCW participants, 51 (25.2%) were colonized with S. *aureus* of which 5 (9.8%) were found to be MRSA. The distribution of *S. aureus* varied between different age groups. Higher frequencies of *S. aureus* and MRSA were found in the age group 21–29 years, but the difference was not statistically significant. The percentage of *S. aureus* and MRSA among HCWs was relatively higher in the surgery department, 11 (21.6%) and 2 (40.0%), respectively; however, this difference was not statistically significant. Similarly, there were no significant associations between isolation of *S. aureus* and years of work experience among HCWs (S1 Table).

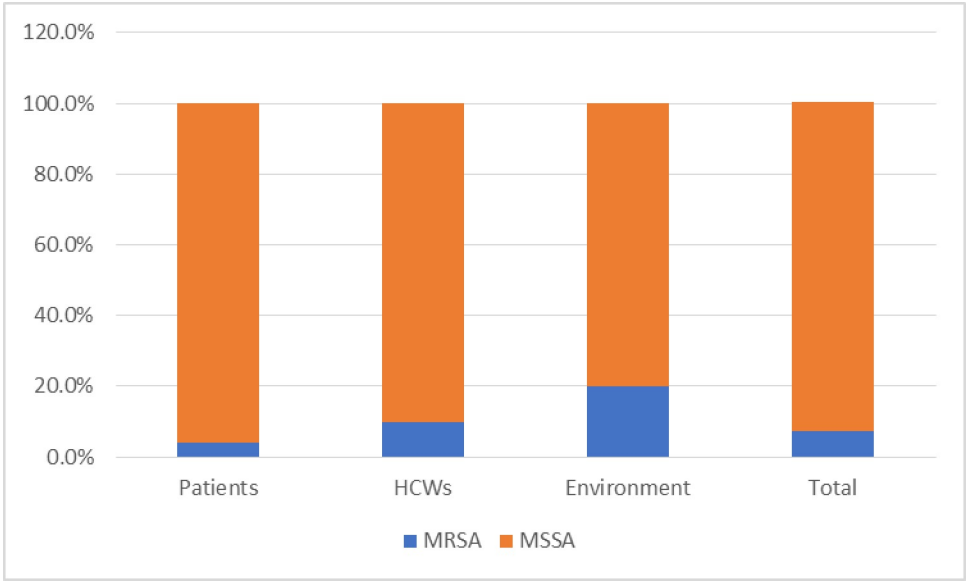

Fig 1. Methicillin-resistant and methicillin-sensitive Staphylococcus aureus by sample source. Abbreviations: MRSA: methicillin resistant; MSSA: methicillin susceptible resistant; HCW: Healthcare worker.

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Out of the 90 environmental samples, 10 (11.1%) yielded *S. aureus* isolates. Two of these isolates (20.0%) were MRSA. One MRSA isolate was recovered from the patient area, while the other was from the staff area.

Antimicrobial resistance

Antimicrobial susceptibility testing was performed on 134 *S. aureus* isolates: 73 from patients, 51 from HCWs, and 10 from environment (Table 3). One hundred eighteen 118 (88.1%) were resistant to ampicillin and penicillin; 70 (52.2%) were resistant to trimethoprim sulfamethoxazole (SXT); 28 (20.9%) were resistant to erythromycin; 22 (16.4%) were resistant to tetracycline; 21 (15.7%) were resistant to clindamycin; 5 (3.7%) were resistant to ciprofloxacin; and 2 (1.5%) were resistant to ceftaroline. A total of 11 (8.2%) isolates were resistant to oxacillin, but one isolate was negative for methicillin resistance using the cefoxitin test. A total of 10 (7.4%) isolates were therefore, identified as MRSA (i.e., the resistance was resulting from the presence of *mecA* gene) (Table 3). None of the isolates were resistant to vancomycin, linezolid, daptomycin, nitrofurantoin and high-level mupirocin.

All of the MRSA isolates were resistant to cefotaxime, whereas 7 (70.0%) of the MRSA isolates were also resistant to SXT; 6 (60.0%) were resistant to erythromycin; 5 (50.0%) were resistant to ciprofloxacin; 4 (40.0%) were resistant to levofloxacin; 3 (30.0%) were resistant to

Table 3. Antimicrobial resistance patterns of methicillin-resistant and methicillin sensitive *Staphylococcus aureus*.

Antibiotics	Total (N = 134)	Total MSSA (N = 124)	Total MRSA (N = 10)
	Resistance n (%)	Resistance n (%)	Resistance n (%)
Ampicillin	118 (88.1)	108 (87.1)	10 (100.0)
Cefotaxime	8 (5.9)	0 (0.0)	10 (1000.0)
Cefoxitin	10 (7.4)	0 (0.0)	10 (100.0)
Ceftaroline	2 (1.5) *	0 (0.0)	2 (20.0) *
Ciprofloxacin	5 (3.7)	0 (0.0)	5 (50.0)
Clindamycin	21 (15.7) *	18 (14.5)	3 (30.0)
Daptomycin	0 (0.0)	0(0.0)	0 (0.0)
Erythromycin	28 (20.9)	22 (17.7)	6 (60.0)
ICR	15(11.2)	14(11.3)	1 (10.0)
Gentamicin	2 (1.5)	0 (0.00)	2 (20.0)
Levofloxacin	6 (4.5) *	2 (1.7)	4 (40.0) *
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)
Moxifloxacin	4 (3.0) *	0 (0.0)	4 (40.0)
Mupirocin HL	0 (0.0)	0 (0.0)	0 (0.00)
Nitrofurantoin	0 (0.0)	0 (0.0)	0 (0.0)
Oxacillin	11 (8.2)	1 (0.8)	10 (100.0)
Penicillin G	118 (88.1)	108 (87.1)	10 (100.0)
Rifampicin	1 (0.75)	0 (0.0)	1 (10.0)
SXT	70 (52.2)	63 (50.8)	7 (70.0)
Teicoplanin	4 (3.0)	4 (3.2)	0 (0.0)
Tetracycline	22 (16.4) *	8 (15.3) *	3 (30.0)
Tigecycline	10 (7.5) *	9 (7.3) *	1 (10.0)
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; HCW, healthcare worker; Environ., environmental; ICR, inducible clindamycin resistance HL, high level mupirocin resistance; SXT: Trimethoprim-Sulfamethoxazole; *Intermediate resistance was classified as resistant.

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Table 4. Antimicrobial resistance patterns of *Staphylococcus aureus* strains from patients, Health Care Workers, and hospital environment.

Antibiotics	Patient (N = 73) Resistance n (%)	HCW (N = 51) Resistance n (%)	Environment (N = 10) Resistance n (%)
Ampicillin	67 (91.8)	41 (80.4)	10 (100.0)
Cefotaxime	3 (4.1)	5 (9.8)	2 (20.0)
Cefoxitin	3 (4.1)	5 (9.8)	2 (20.0)
Ceftaroline	1 (1.4) *	1 (2.0) *	0 (0.0)
Ciprofloxacin	1 (1.4)	2 (3.9)	2 (20.0)
Clindamycin	8 (11.0)	10 (19.6)	3 (30.0) *
Daptomycin	0 (0.0)	0 (0.0)	0 (0.0)
Erythromycin	13 (17.8)	12 (23.5)	3 (30.0)
ICR	7(9.6)	8(15.6)	0(0.0)
Gentamicin	0 (0.00)	1 (2.0)	1 (10.0)
Levofloxacin	1 (1.4)	2 (3.9) *	3 (30.0)
Linezolid	0 (0.0)	0 (0.0)	0 (0.0)
Moxifloxacin	1 (1.4)	1 (2.0)	2 (20.0)
Mupirocin HL	0 (0.0)	0 (0.0)	0 (0.0)
Nitrofurantoin	0 (0.0)	0 (0.0)	0 (0.0)
Oxacillin	3 (4.1)	6 (11.8)	2 (20.0)
Penicillin G	66 (90.4)	42 (82.4)	10 (100.0)
Rifampicin	0 (0.0)	0 (0.0)	1 (10.0)
SXT	46 (63.0) ‘	22 (43.1)	2 (20.0)
Teicoplanin	4 (5.48)	0 (0.0)	0 (0.0)
Tetracycline	12 (16.4)	10 (19.6) *	0 (0.0)
Tigecycline	7 (9.6)	3 (5.9) *	0 (0.0)
Vancomycin	0 (0.0)	0 (0.0)	0 (0.0)

SXT, trimethoprim-sulfamethoxazole

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tetracycline; 2 (20.0) were intermediate resistant to ceftaroline; and 1 (10%) was resistant to rifampicin (Table 3).

Among the 124 (92.6%) MSSA isolates 108 (87.1%) were resistance to benzylpenicillin; 63 (50.8%) were resistant to SXT and 22 (17.7) were resistant to erythromycin. In addition, 18 (14.5%) and 19 (15.3%) isolates were resistance to clindamycin and tetracycline, respectively.

Patients

Of the total 73 *S. aureus* isolates from patients tested for AST, 67 (91.8%) were resistant to ampicillin, and 66 (90.4%) were resistant to penicillin; 46 (63.0%) were resistance to SXT; 12 (16.4%) were resistant to tetracycline; 13 (17.8%) were resistant to erythromycin; 8 (11.0%) were resistant to clindamycin; 4 (5.5%) were resistant to teicoplanin; and 1 (1.4%) was resistant to ceftaroline (Table 4).

Health care worker

Of the total 51 HCW *S. aureus* isolates tested for AST, 41 (80.4%) and 42 (82.4%) were resistant to ampicillin and penicillin respectively; 22 (43.1%) were resistance to SXT; 12 (23.5%) were resistant to erythromycin; 10 (19.6%) resistant to tetracycline; 10 (19.6%) were resistant to clindamycin; 1 (2.0%) was resistant to ceftaroline. Six (11.7%) isolates were resistant to oxacillin, but one was cefoxitin-screening negative. Five (9.8%) isolates were therefore identified as MRSA (Table 4).

Table 5. Association of antimicrobial resistance and sample source.

Antibiotics	Patients		HCW	
	OR	p-value	OR	p-value
Ampicillin	2.724	0.070	-	-
Cefoxitin	0.170	0.074	0.430	0.360
Cefotaxime	0.170	0.074	0.435	0.365
Ceftaroline	-	-	0.694	0.798
Ciprofloxacin	0.056	**0.024**	0.205	0.140
Clindamycin	0.287	0.112	0.569	0.467
Daptomycin	-	-	-	-
Erythromycin	0.506	0.366	0.717	0.665
Gentamicin	-	-	0.180	0.240
Levofloxacin	**0.032**	**0.005**	0.095	**0.019**
Oxacillin	0.171	0.074	0.533	0.486
Penicillin G	2.020	0.194	NA	NA
Tetracycline	0.806	0.650	NA	NA
Tigecycline	1.697	0.460	NA	NA
SXT	6.815	**0.020**	3.034	0.186

Notes: 95% confidence intervals were used

Abbreviations: HCW, Health care worker; OR, odds ratio; SXT, trimethoprim-sulfamethoxazole

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Environment

All of the10 *S. aureus* strain isolated from hospital environment were resistant to both ampicillin and penicillin while 3 (30.0%) were resistant to clindamycin, erythromycin and levofloxacin. None of the environmental isolates were resistant to ceftaroline and tetracycline (Table 4).

Associations between antibiotic resistance and sample sources

Table 5 below shows the association between antimicrobial resistance and sample source (patients vs. healthcare workers in reference to environmental sample) for several different antibiotics. The table includes odds ratios (OR) and p-values for each antibiotic. Significantly high level of resistance was observed in levofloxacin and ciprofloxacin in environmental samples compared to patient's sample. While the odds of *S. aureus* to be resistant to SXT among patients are 6.81times more, that is statically significant.

spa, *mecA*, and *lukF*-PV gene detection

All 134 isolates tested positive for the presence of the *spa* genes, whereas the *mec*A gene was only detected in 10 (7.4%) MRSA isolates. The *lukF-PV* gene was detected in 92/134 (68.6%) of the *S. aureus* isolates, including in 62 (85.0%), 26 (51.0%) and 4 (40.0%) of the patients, healthcare worker, and environmental isolates, respectively.

Four (40%) of the 10 *mecA* positive MRSA isolates possessed the *lukF-PV* gene, compared to 87/124 (70.2%) of MSSA isolates. Among the latter, 60/87 (69.0%) were isolated from patients, while 25/87 (28.7%) were isolated from health care workers and 2 (2.3%) from environmental samples. Significant associations were observed between isolates from patients and the presence of the *lukF-PV* gene in reference to environmental samples (Table 6). *lukF-PV* gene positive isolates had a 4.54 times higher probability of being SXT-resistant; while oxacillin-resistant isolates were less common among isolates that tested positive for *lukF-PV* gene

Table 6. Association of resistant isolate and, *lukF-PV* prevalence, and specimen source, *lukF-PV* prevalence and isolate department, MRSA and specimen source.

All samples antimicrobial resistance and PVL association			
Antibiotics	**PVL**	**OR**	**p-value**
Cefoxitin	4(40.0)	0.280	0.062
Ampicillin	80(67.8)	0.950	0.940
Cefotaxime	4(40.0)	0.280	0.062
Ceftaroline	1(50.0)	0.470	0.593
Ciprofloxacin	2(40)	0.278	0.171
Clindamycin	13(61.9)	0.729	0.522
Erythromycin	15(53.6)	0.456	0.071
Levofloxacin	3(50.0)	0.454	0.347
Oxacillin	4(36.4)	**0.236**	**0.028**
Penicillin G	79(66.9)	0.675	0.519
Tetracycline	16(72.7)	1.316	0.597
Tigecycline	8(80.0)	1.975	0.402
SXT	58(82.8)	**4.540**	**<0.001**
Association of PVL and sample source			
Sample source	**PVL**	**OR**	**p-value**
Patients	62(84.9)	13.150	**0.001**
HCWs	26(50.9)	2.420	1.190
Environment	1	1	1

Notes: 95% confidence intervals were used.

Abbreviations: HCW, Health care worker; OR, odds ratio; significant *p*-value (< = 0.05) are shown in boldface

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(Table 6). However, no associations were found between *lukF-PV* prevalence and departmental location.

Discussion

The emergence and persistent spread of drug-resistant *S. aureus* have become one of the most frightening problems facing the world today. In the present study, we found that the majority of isolates were MSSA; nevertheless, these were resistant to commonly prescribed oral antibiotics such as penicillin, SXT, tetracycline, erythromycin and clindamycin resulting in limited treatment options for outpatients. In addition, over two thirds of the isolates harbored the *lukF*-PV gene encoding PVL, indicating increase virulence in the isolates.

Association between *S. aureus* / MRSA infection and host attributes such as age and gender have been searched for in the present study. The most frequent age ranges for female patients were 26 to 39 years old and for male it was under 1 year of age. With the exception of four blood samples, most of the children under a year old were from outpatient and had pus or abscesses. Since the children were not hospitalized and the majority of their *S. aureus* infections were community associated, an outbreak in the hospital was not likely the source of the children's infection.

Male children displayed a slightly higher prevalence of *S. aureus* infection among other age groups. Overall, there was a slightly higher percent of female patients in our study, in contrast to other studies that have reported a higher prevalence of *S. aureus* infection in males [7]. Majority of female patients were in the reproductive age and some of them were breast feeding (data not shown). *S. aureus* transmission from mother to child has been reported in different studies [20, 21]. Given the high infection rate among young mothers and infants found in the

present study, further studies are needed in our patient population to conclude the risk of mother-to-child transmission of *S. aureus*.

S. aureus exhibit many virulence factors that are important or necessary for its survival and ability to infect humans. One of the key components of *S. aureus* pathogenicity that helps it avoid human immunological reactions is staphylococcal protein A, [22]. In this study, the spa gene was found in every isolate; however, in other studies, the difference in primer binding sites caused up to 3% of the isolates not to detect the gene [23]. Panton-Valentine Leukocidin (PVL) is another virulence gene expressed by *S. aureus* and is known to be associated with severe form of infection. It is usually associated with skin and soft tissue infection and sepsis [24, 25]. In this study over two-third of the isolates possessed PVL gene and this is in agreement with previous study conducted in Ethiopia indicating that there is a high prevalence virulent *S. aureus* strains encoding *pvl* gene in the county [26].

S. aureus may carry the *mecA* gene, which confers resistance to methicillin, penicillin, and other drugs that resemble penicillin. In this study the detection of MRSA in patients was very low compared to other studies conducted in Ethiopia [12, 13]. Other studies utilizing molecular techniques also revealed very low frequencies of MRSA cases in Ethiopia [14, 27]. This may be because the majority of the study conducted used Kirby-Bauer disc diffusion method by cefoxitin screening or oxacillin test (which needs E- test for confirmation and most of the laboratories do not have E tests). Similar condition was reported in other East Africa country [28].

Interestingly, the age range of the majority of HCWs was between 21 and 29 years of age, indicating a young workforce. This is consistent with the report that showed the majority of healthcare professionals in Ethiopia were under 35 years old [29]. This could be the cause of the lack of significant association between MRSA carriage and age group. The percentage of *S. aureus* identification was comparable to other studies in Ethiopia [30, 31]. The HCWs' department and year of experience had no significant association with the presence of MRSA. In contrast, other studies investigating the nasal carriage of *S. aureus* and MRSA, reported significant associations with one or more of the aforementioned factors [31, 32]. Similarly, there was no statistically significant association between the hospital ward and the sample collection area among environmental isolates of MRSA.

In environmental samples, the frequency of MRSA was slightly higher. In addition, fluoroquinolones have shown significantly high level of resistance in environmental samples compared to other samples sources. This may be because the bacteria in a hospital environment are more likely to develop antibiotic resistance due to high use [33, 34]. A study conducted elsewhere reported high MRSA contamination in hospital surfaces during no outbreak periods [35]. A study conducted in another hospital in Addis Ababa reported a comparable MRSA colonization rate of 9.8% among health care workers [31].

In this investigation, all *S. aureus* isolates were susceptible to antibiotics such as daptomycin, linezolid, nitrofurantoin, and vancomycin. These findings were in line with those of other East African studies [14, 36, 37]. For instance, linezolid is only authorized for use in certain centers and is only designated for the treatment of multidrug-resistant tuberculosis [38]. Other antibiotics, such as daptomycin, are not included in the national drug list [38].

Since every isolate was responsive to the previously listed antibiotics, daptomycin and vancomycin are prescribed for serious infections such as bacteremia/ osteomyelitis caused by MRSA or for those with penicillin allergy who have MSSA and are unable to take oxacillin. It ensures that patients with severe invasive *S. aureus* infections may still have recourse to potent antibiotics. By contrast, a substantial percentage of regularly used antibiotics, including oral medications such as penicillin, SXT, and tetracycline, demonstrated high levels of resistance. Studies carried out elsewhere in Ethiopia have revealed a similar resistance profile [11, 14]. This is likely connected to self-medication and overuse of antibiotics in community settings

[37, 39]. There are also reports indicating that many antibiotics are prescribed empirically and unnecessarily [40, 41].

An isolate from HCW sample displayed resistance to oxacillin but was susceptible to cefoxitin. Cefoxitin is more reliable indicator of *mecA* gene carriage, and molecular analysis confirmed that this isolate did not harbor the *mecA* gene. This finding highlights that resistance to oxacillin is mediated by resistance mechanisms other than *mecA* [42].

Despite the fact that ceftaroline has been shown to have increased activity against MRSA in many countries globally [14, 43, 44], it is concerning that we have identified ceftaroline resistant isolates. Especially because this antibiotic is not included in Ethiopia's list of essential medications and is not available at SPHMMC [38]. Ceftaroline may be available in some hospitals in Addis Ababa and this resistance could be derived from selection pressure. It is also alarming that of the two MRSA isolates with intermediate resistance to ceftaroline, one originated from a patient and the other from a healthcare provider.

The virulence gene *lukF-PV* was present in around two-thirds of the isolates, including 40% of the MRSA isolates. When compared isolates from other sources, patient sample isolates displayed greater levels of resistance to SXT which demonstrated statistically significant associations. The detection of the virulence gene *(lukF-PV)* was relatively higher across all sample sources, with patient samples displaying the most significant statistical correlation. This may be linked to the possibility that isolates that produce infection are more virulent as compared to strains identified in environment. A study from East Africa suggested that the *pvl* toxin may be increasing in prevalence among clinical isolates [45].

Whereas a previous study in Ethiopia did not find the *lukF-PV* gene in MRSA isolates, this study observed the *lukF-PV* gene in *mecA* positive isolates [46]. Similar reports from other East African countries suggest that MRSA strains harboring PVL represent significant pathogenic strains in patients with skin and soft tissue infections [47].

Conclusion

Although, some of the antibiotics such as vancomycin, linezolid and daptomycin were complete susceptible to *S. aureus*, there is higher resistance against common antibiotics such as penicillin, trimethoprim sulfamethoxazole and erythromycin and clindamycin. Ceftaroline resistance is concerning as well, and since testing for resistance is not regularly done, additional study is required to determine the true prevalence of the resistance. The prevalence of MRSA found in patients, healthcare workers, and the environment was comparatively low. The detection of MSSA or MRSA was shown to have no association with sample source, sample collection department, gender and age. Moreover, patient isolates had significant association with high prevalence of *lukF-PV* gene. These findings demonstrate the emergence of antimicrobial resistance to new drugs and the prevalence of virulent *S. aureus* strains in the study site. Further studies to identify potential transmission links and improved infection prevention and control and environmental cleaning are needed to prevent spread. In addition, we recommend enforcing proper use and regulation of antibiotics in Ethiopia.

Supporting information

S1 Table. Demographics of health care workers and positive isolates from each sample and correlation of the age, sex, department, work experience, profession and level of education to isolation of *S. aureus*/MRSA.
(DOCX)

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References

1. Kluytmans J, van Belkum A, Verbrugh H. Nasal Carriage of Staphylococcus aureus: Epidemiology, Underlying Mechanisms, and Associated Risks. Vol. 10. 1997. https://doi.org/10.1128/CMR.10.3.505 PMID: 9227864
2. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. Staphylococcus aureus nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Vol. 9, Frontiers in Microbiology. Frontiers Media S.A.; 2018. https://doi.org/10.3389/fmicb.2018.02419 PMID: 30349525
3. Laux C, Peschel A, Krismer B. Staphylococcus aureus Colonization of the Human Nose and Interaction with Other Microbiome Members. Microbiol Spectr. 2019 Apr 12; 7(2). https://doi.org/10.1128/microbiolspec.GPP3-0029-2018 PMID: 31004422
4. Popovich KJ, Green SJ, Okamoto K, Rhee Y, Hayden MK, Schoeny M, et al. MRSA Transmission in Intensive Care Units: Genomic Analysis of Patients, Their Environments, and Healthcare Workers. Clinical Infectious Diseases. 2021 Jun 1; 72(11):1879–87. https://doi.org/10.1093/cid/ciaa731 PMID: 32505135
5. Conceição T, Santos Silva I, De Lencastre H, Aires-De-Sousa M. Staphylococcus aureus nasal carriage among patients and health care workers in são tomé and príncipe. Microbial Drug Resistance. 2014 Feb 1; 20(1):57–66.
6. Kobayashi SD, Malachowa N, Deleo FR. Pathogenesis of Staphylococcus aureus abscesses. American Journal of Pathology [Internet]. 2015; 185(6):1518–27. Available from: https://doi.org/10.1016/j.ajpath.2014.11.030 PMID: 25749135
7. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. Staphylococcus aureus infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28 (3):603–61. https://doi.org/10.1128/CMR.00134-14 PMID: 26016486
8. Nguyen AT, Tallent SM. From commensal to consumer: Staphylococcus aureus toxins, diseases, and detection methods. Vol. 101, Journal of AOAC International. Oxford University Press; 2018. p. 1127–34. https://doi.org/10.5740/jaoacint.17-0366 PMID: 29216934
9. 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. International Journal of Infectious Diseases. 2013 Feb; 17(2). https://doi.org/10.1016/j.ijid.2012.09.006 PMID: 23092752

10. Mohamadou M, Essama SR, Essome MCN, Akwah L, Nadeem N, Kamga HG, et al. High prevalence of Panton-Valentine leukocidin positive, multidrug resistant, Methicillin-resistant Staphylococcus aureus strains circulating among clinical setups in Adamawa and Far North regions of Cameroon. PLoS One. 2022 Jul 1; 17(7 July). https://doi.org/10.1371/journal.pone.0265118 PMID: 35802616

11. Mama M, Aklilu A, Misgna K, Tadesse M, Alemayehu E. Methicillin- and Inducible Clindamycin-Resistant Staphylococcus aureus among Patients with Wound Infection Attending Arba Minch Hospital, South Ethiopia. Int J Microbiol. 2019;2019. https://doi.org/10.1155/2019/2965490 PMID: 31065270

12. Dilnessa T, Bitew A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. BMC Infect Dis. 2016 Aug 9; 16(1). https://doi.org/10.1186/s12879-016-1742-5 PMID: 27506613

13. Deyno S, Toma A, Worku M, Bekele M. Antimicrobial resistance profile of staphylococcus aureus isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. BMC Pharmacol Toxicol. 2017; 18(1):1–7.

14. Verdú-Expósito C, Romanyk J, Cuadros-González J, TesfaMariam A, Copa-Patiño JL, Pérez-Serrano J, et al. Study of susceptibility to antibiotics and molecular characterization of high virulence Staphylococcus aureus strains isolated from a rural hospital in Ethiopia. PLoS One. 2020; 15(3):1–17.

15. Shibabaw A, Abebe T, Mihret A. Antimicrobial susceptibility pattern of nasal Staphylococcus aureus among Dessie Referral Hospital health care workers, Dessie, Northeast Ethiopia. 2014 [cited 2019 Oct 18]; Available from: https://doi.org/10.1016/j.ijid.2014.03.1386 PMID: 24813590

16. Weese JS, DaCosta T, Button L, Goth K, Ethier M, Boehnke K. Isolation of Methicillin-Resistant Staphylococcus aureus from the Environment in a Veterinary Teaching Hospital. J Vet Intern Med. 2004 Jul; 18(4):468–70. https://doi.org/10.1892/0891-6640(2004)18<468:iomsaf>2.0.co;2 PMID: 15320581

17. Phoenix™ M50 Automated Microbiology System User's Manual Change History. 2021. https://dmec.moh.gov.vn/documents/10182/35406975/upload_00006458_1664954917079.pdf?version=1.0&fileId=35422496

18. QIAGEN. QIAamp DNA Mini and Blood Mini Handbook. Qiagen. 2016;(5):1–72.

19. Sample to Insight Microbial DNA qPCR Instructions for Use For real-time PCR-based profiling/detection of microbial species, antibiotic resistance genes or virulence factor genes Microbial DNA qPCR Array Microbial DNA qPCR Assay/Multi-Assay Kits 2. 2022.

20. Lin J, Yao Z. Maternal-infant correlation of multidrug-resistant Staphylococcus aureus Carriage: A prospective cohort study. Front Pediatr. 2018; 6. https://doi.org/10.3389/fped.2018.00384 PMID: 30568937

21. Schaumburg F, Alabi AS, Mombo-Ngoma G, Kaba H, Zoleko RM, Diop DA, et al. Transmission of Staphylococcus aureus between mothers and infants in an African setting. Clinical Microbiology and Infection. 2014; 20(6).

22. Votintseva A. A, Fung R., Miller R. R, Knox K., Godwin H., Wyllie D. H, Bowden R., Crook D. W, and Walker A S. (2014) Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire. Votintseva et al. BMC Microbiology 2014, 14:63 http://www.biomedcentral.com/1471-2180/14/63

23. Haggag MG, Aboelnour AE, Al-Kaffas M. MRSA screening and spa gene detection in isolates from healthcare workers at ophthalmology hospital in Egypt. Bull Natl Res Cent. 2019 Dec; 43(1).

24. Ahmada N. I., Yeana C. Y., Fooa P. C., Mohamad Safiee A. W., Hassana S. A., Prevalence and association of Panton-Valentine Leukocidin gene with the risk of sepsis in patients infected with Methicillin Resistant Staphylococcus aureus. Journal of Infection and Public Health 13 (2020) 1508–1512 https://doi.org/10.1016/j.jiph.2020.06.018 PMID: 32653480

25. Bhatta D. R.1, Cavaco L. M., Nath G., Kumar K., Gaur A., Gokhale S.4 and Dwij R. Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: a matter of concern for community infections (a hospital based prospective study) Bhatta1. BMC Infectious Diseases (2016) 16:199 https://doi.org/10.1186/s12879-016-1531-1 PMID: 27179682

26. Verdú-Expósito C, Romanyk J, Cuadros-González J, TesfaMariam A, Copa-Patiño JL, Pérez-Serrano J, et al. Study of susceptibility to antibiotics and molecular characterization of high virulence Staphylococcus aureus strains isolated from a rural hospital in Ethiopia. PLoS One. 2020; 15(3):1–17.

27. Eyasu T, Tesfu K, Daniel A, Haile A, Thomas S, Pamela RFA, et al. Phenotypic and genotypic characterization of Staphylococcus aureus isolates recovered from bovine milk in central highlands of Ethiopia. Afr J Microbiol Res. 2015; 9(44):2209–17.

28. Omuse G, Kabera B, Revathi G. Low prevalence of methicillin resistant as determined by an automated identification system in two private hospitals in Nairobi, Kenya: A cross sectional study. BMC Infect Dis. 2015 Feb 6; 14(1).

29. Feysia B, Herbst CH, Lemma W, Soucat A. A W O R L D B A N K S T U D Y The Health Workforce in Ethiopia. https://www.exemplars.health/-/media/files/egh/resources/underfive-mortality/ethiopia/feysia_the-health-workforce-in-ethiopia-addressing-remaining-challenges.pdf?la=en

30. Reta A, Mengist A, Tesfahun A. Nasal colonization of methicillin resistant Staphylococcus aureus in Ethiopia: A systematic review and meta-analysis. Ann Clin Microbiol Antimicrob [Internet]. 2019; 18 (1):1–12. Available from: https://doi.org/10.1186/s12941-019-0324-y PMID: 31488199

31. Desta K, Aklillu E, Gebrehiwot Y, Enquselassie F, Cantillon D, Al-Hassan L, et al. High Levels of Methicillin-Resistant Staphylococcus aureus Carriage Among Healthcare Workers at a Teaching Hospital in Addis Ababa Ethiopia: First Evidence Using mecA Detection. Infect Drug Resist. 2022; 15:3135–47. https://doi.org/10.2147/IDR.S360123 PMID: 35747330

32. Legese H, Kahsay AG, Kahsay A, Araya T, Adhanom G, Muthupandian S, et al. Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus among healthcare workers in Adigrat and Wukro hospitals, Tigray, Northern Ethiopia. BMC Res Notes. 2018 Apr 23; 11(1). https://doi.org/10.1186/s13104-018-3353-2 PMID: 29685170

33. Rajamohan G, Srinivasan VB, Gebreyes WA. Molecular and functional characterization of a novel efflux pump, AmvA, mediating antimicrobial and disinfectant resistance in Acinetobacter baumannii. Journal of Antimicrobial Chemotherapy. 2010 Jun 23; 65(9):1919–25. https://doi.org/10.1093/jac/dkq195 PMID: 20573661

34. Li Y, Song Y, Huang Z, Mei L, Jiang M, Wang D, et al. Screening of Staphylococcus aureus for Disinfection Evaluation and Transcriptome Analysis of High Tolerance to Chlorine-Containing Disinfectants. Microorganisms. 2023 Feb 1; 11(2). https://doi.org/10.3390/microorganisms11020475 PMID: 36838440

35. van Balen J, Bottichio L, Stevenson K, Wang SH, Nava-Hoet R, Hoet AE. Understanding the introduction and circulation of environmental methicillin-resistant Staphylococcus aureus in a large academic medical center during a nonoutbreak, year-long period. Am J Infect Control. 2016 Aug 1; 44(8):925–30. https://doi.org/10.1016/j.ajic.2016.02.039 PMID: 27480895

36. Wangai FK, Masika MM, Maritim MC, Seaton RA. Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa: Red alert or red herring? BMC Infect Dis. 2019 Jul 9; 19(1). https://doi.org/10.1186/s12879-019-4245-3 PMID: 31288757

37. Gebrekirstos NH, Workneh BD, Gebregiorgis YS, Misgina KH, Weldehaweria NB, Weldu MG, et al. Non-prescribed antimicrobial use and associated factors among customers in drug retail outlet in Central Zone of Tigray, northern Ethiopia: A cross-sectional study. Antimicrob Resist Infect Control. 2017; 6 (1):1–10. https://doi.org/10.1186/s13756-017-0227-7 PMID: 28670450

38. Ministry of Health/Ethiopian Food and Drug Authority. http://www.fmhaca.gov.et/wp-content/uploads/2020/12/EML-sixth-edition.pdf

39. Sisay M, Mengistu G, Edessa D. Epidemiology of self-medication in Ethiopia: A systematic review and meta-analysis of observational studies. BMC Pharmacol Toxicol. 2018; 19(1):1–12.

40. Fenta T, Engidawork E, Amogne W, Beyene Berha A. Evaluation of current practice of antimicrobial use and clinical outcome of patients with pneumonia at a tertiary care hospital in Ethiopia: A prospective observational study. PLoS One. 2020 Jan 1; 15(1).

41. Gebretekle GB, Haile Mariam D, Abebe Taye W, Mulu Fentie A, Amogne Degu W, Alemayehu T, et al. Half of Prescribed Antibiotics Are Not Needed: A Pharmacist-Led Antimicrobial Stewardship Intervention and Clinical Outcomes in a Referral Hospital in Ethiopia. Front Public Health. 2020 Apr 9; 8.

42. S R, V P, D'Souza AO, Vinod R. Comparison of Phenotypic and Genotypic Characterization Methods for the Detection of Methicillin-Resistant Staphylococcus Aureus. Cureus. 2022 Mar 22; https://doi.org/10.7759/cureus.23396 PMID: 35481290

43. Karlowsky JA, Hackel MA, Bouchillon S I. K, Lowman W, Kotb REM, Mohamed N, et al. In vitro activity of ceftaroline against bacterial pathogens isolated from patients with skin and soft tissue and respiratory tract infections in the Middle East and Africa: AWARE global surveillance programme 2015–2018. J Glob Antimicrob Resist. 2021 Mar 1; 24:249–56. https://doi.org/10.1016/j.jgar.2020.12.013 PMID: 33373731

44. Piérard D, Stone GG. In vitro activity of ceftaroline and comparators against bacterial isolates collected globally from patients with skin infections. J Glob Antimicrob Resist. 2021 Sep 1; 26:4–10. https://doi.org/10.1016/j.jgar.2021.04.020 PMID: 34022417

45. Moremi N, Claus H, Vogel U, Mshana SE. The role of patients and healthcare workers Staphylococcus aureus nasal colonization in occurrence of surgical site infection among patients admitted in two centers in Tanzania. Antimicrob Resist Infect Control. 2019 Jun 17; 8(1).

46. Ibrahim RA, Berhe N, Mekuria Z, Seyoum ET, Balada-Llasat JM, Abebe T, et al. Antimicrobial Resistance and Virulence Gene Profile of Clinical Staphylococcus aureus: A Multi-Center Study from Ethiopia. Infect Drug Resist. 2023; 16:4835–44. https://doi.org/10.2147/IDR.S419577 PMID: 37520455

47. Iliya S, Mwangi J, Maathai R, Muriuki M, Wainaina C. Molecular Detection of Panton Valentine Leukocidin Toxin in Clinical Isolates of Staphylococcus aureus from Kiambu County, Kenya. Int J Microbiol. 2020; 2020.