International Journal of Research in Medical Sciences
Gebremariam T et al. Int J Res Med Sci. 2024 Nov;12(11):4011-4021
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20243346

Original Research Article

Antibiogram profile and phenotypic characteristics of clinical isolates of *Staphylococcus aureus* at Ayder Referral Hospital, Tigray, Ethiopia

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Received: 25 August 2024
Revised: 16 October 2024
Accepted: 17 October 2024

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ABSTRACT

Background: *Staphylococcus aureus* is a notorious pathogen known for its ability to form biofilms, contributing to a spectrum of infections ranging from minor skin abscesses to life-threatening bloodstream infections. The biofilm-forming capability of *S. aureus* complicates treatment and contributes to resistance to antimicrobial agents. This study investigates the antimicrobial susceptibility profiles and biofilm-forming capacities of clinical isolates of *S. aureus*.
Methods: A total of 118 clinical isolates of *S. aureus* were collected and subjected to antimicrobial susceptibility testing using the disk diffusion method against a panel of 17 antibiotics. Biofilm formation was assessed quantitatively using the tissue culture plate (TCP) method, which categorizes biofilm production into strong, moderate, or weak/non-based on optical density measurements.
Results: The results revealed a high resistance rate to ampicillin (71%), while resistance to linezolid was observed in only 2.54% of isolates. Notably, 49.15% of the isolates were identified as methicillin-resistant *S. aureus* (MRSA). Among the isolates, 64.4% were biofilm producers, with 64.5% of these being strong biofilm formers and 9.2% classified as weak biofilm formers. A significant correlation was found between antimicrobial resistance and biofilm formation, with 43 (74%) of the MRSA isolates being strong biofilm formers. This association underscores the challenge posed by biofilm-associated resistance in *S. aureus* infections.
Conclusions: Strategies to combat biofilm-associated infections, including development of novel antimicrobial agents and biofilm-disrupting compounds, are urgently needed to mitigate the public health impact of *S. aureus* infections.

Keywords: Antibiogram, Biofilm, Hospital infections, Methicillin-resistant *Staphylococcus aureus*, Ayder hospital

INTRODUCTION

S. aureus is a normal flora and a pathogen causing various illnesses, ranging from minor skin infections to life-threatening diseases. [1-2] *S. aureus* demonstrates a niche preference for the anterior nares, especially in adults, and the nasal carrier rates can vary from 10% to 40% in both the community and hospital environment.[2] The nasal carriage of *S. aureus* is one means of persistence and spread of multi-drug resistant (MDR)

Staphylococci, especially methicillin-resistant *S. aureus* (MRSA).[2,3]

S. aureus is a group of microorganisms covered by an exopolysaccharide matrix or biofilms.[4] Biofilm formation is commonly considered to occur in different stages, prosper upon moist or wet surfaces, and establish itself in such environments for a very long time.[4,5] The Ica gene codes for intracellular adhesion (ICA) may also code for TS/A and are required for biofilm production.[5,6] The microbes are surrounded by extracellular polymeric substances (EPS) after developing into microcolonies. Intercellular signaling or quorum sensing (QS) occurs in the EPS matrix at this point. One could promote or inhibit biofilm formation by manipulating EPS-based phenotypes and the QS system.[7] Microorganisms growing in a biofilm are more resistant to antimicrobials than planktonic cells.[6,7] Biofilm-producing *S. aureus* frequently colonizes catheters and medical devices and may cause foreign body-related topical and systemic infections.[8,9] Studies show that *S. aureus* has an Ica locus, encoding for function of intracellular adhesion and biofilm formation.[8,9] According to a recent public statement from the National Institute of Health (NIH), more than 60% of all infections are secondary to biofilm-forming microorganisms.[10-12]

A study in northern Ethiopia showed that *S. aureus*-induced surgical site infection rate accounted for 10.2% and was the dominantly leading bacterial pathogen responsible for the surgical site and other types of infections.[2,14,16] Antimicrobial resistance (AMR) in MRSA is associated with the acquisition of a large mobile genetic element called *Staphylococcal* cassette chromosome (SCCmec), which carries the central determinant for a broad-spectrum beta-lactam resistance encoded by *mecA* or *mecC* genes.[17,18] MRSA can spread unless strict infection prevention and control strategies are implemented.[19]

The possible public health threat due to AMR is high because antimicrobial agents can easily be dispensed without prescription in most developing countries, including Ethiopia.[13,20,21] In addition, lack of consistent surveillance of AMR, inadequate antibiogram profiling, poor laboratory diagnostic capacity, and poor infection prevention and control techniques by health facilities and individuals lead to the emergence and spread of AMR.[22,23] A study conducted in Addis Ababa, the capital city of Ethiopia, reported an isolation rate of 14.3% for *S. aureus* from clinical specimens, of which over 50% were MDR and 17.5% were MRSA.[24-26] Inadequate information is available on occurrence antimicrobial susceptibility and the antibiogram profile of *S. aureus* in patients with different infections in Tigray, parts of Amhara and coastal areas of Eritrea. This study aimed to assess the antibiogram profile and phenotypic characteristics of clinical isolates of *S. aureus* obtained from patients and a corresponding nasal swab from medical staff attending these patients in Ayder Referral Hospital (ARH), Tigray (north Ethiopia).

METHODS

Study setting, design, and subjects

This study was conducted in (ARH). ARH is the largest tertiary and referral hospital in Tigray, and has abed capacity of 500 that serves approximately 3.5 million patients annually.

The study employed a hospital-based cross-sectional design. Hospitalized patients were recruited from various units, representing a diverse population in terms of infection histories, age groups, and sex. Medical staff involved in the care of these patients were also included in the study. Recruitment was conducted using a convenience sampling method, ensuring a broad representation of the hospital population.

Sampling and eligibility

The sample size was calculated using the single proportion formula, assuming a prevalence of 50%, a precision of 5%, a 95% confidence level (Z=1.96), and a margin of error (E=0.05). An additional 10% was added to account for potential non-responses, resulting in an estimated sample size of 430. Samples were collected from various wards and outpatient departments (OPDs) using a convenience sampling method. Exclusion criteria included patients with incomplete data, those unwilling to provide consent, and medical staff who were not actively involved in patient care or were present for only a short period. To minimize duplication and ensure data quality, only the first bacterial isolate of a given species per patient, per admission period, or per medical staff member was included in the analysis.

Specimen collection and S. aureus isolation

A total of 430 specimens (113 blood, 138 nasal swabs, and 179 pus) were collected from patients and medical staff attending patients from different units: Surgical ward (nasal swab, 85; pus, 66); pediatric ward (blood, 41; nasal swab, 18; pus, 58); medical ward (blood, 13; nasal swab, 34; pus, 22); and outpatient department (blood, 8; nasal swab, 26; pus, 53). The specimens were cultured for the isolation of *S. aureus* using standard laboratory techniques. For the specimen taken from the wound, a sterile cotton swab was moistened with normal saline and rotated three times on the wound surface. Specimens from nasal swabs were placed in test tubes containing 10 ml of sterile tryptone soya broth (TSB) (Oxoid Limited, UK). Specimens were then transported to the Microbiology Laboratory of Mekelle University within 1 hour of collection and immediately processed.[9,17,26] The specimens were then incubated at 35°C for 24 hours in TSB. A loopful of the suspension was streaked into mannitol salt agar (Oxoid, Basingstoke, Hampshire,

England), and the plates were incubated at 35°C for 24 hours. Blood was collected in a culture bottle. Fresh blood 20 ml (for adults) and 2-10 ml (for others, according to their age and weight) was typically drawn through venipuncture using a needle and directly transferred to a blood culture bottle containing a growth medium (TSB and brain heart infusion; BHI), which encourages microorganisms to multiply, and an anticoagulant (sodium polyanethol sulfonate, SPS), SPS is the most frequently used anticoagulant as it does not interfere with the growth of most organisms. The collected blood was buffered with 1/50 volume of 1 M HEPES (Sigma-Aldrich h4034), supplemented with 2 mg/l glucose and 48 µg/l adenine. Overnight cultured isolates were diluted 1:1000 in lysogeny broth (LB) medium, spiked into supplemented blood (typically 4 mL), and incubated at 37°C with gentle shaking anaerobically.[8,17,26]

Mannitol salt agar (MSA, 7.5%) was used for selectively and differentially recovering isolates of *S. aureus* (which appeared as yellow on this agar; coagulase-negative *Staphylococci* retained the color of the agar, red). The bacterial colonies with distinctive characteristics of *S. aureus* (i.e., colonies with golden yellow pigmentation) were subjected to subsequent biochemical tests involving Gram stain, catalase, and coagulase for confirmation. *S. aureus* (ATCC25923) was used as a reference strain for quality control purposes.[17,26]

Antimicrobial susceptibility testing

Antimicrobial susceptibility test for *S. aureus* was carried out against a panel of 17 antimicrobials with the Kirby Bauer disc diffusion method using Mueller Hinton agar (MHA) (Oxoid,Basingstoke,England), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Oxoid, Basingstoke, England).[17,26-27] The bacterial culture was grown in TSB at 35°C for 4-5 hours and the inoculum density was adjusted with 0.5 McFarland standard.[17,27] A sterile cotton swab was dipped into the suspension and pressed against the sides of the tube to avoid excess inoculum. The inoculum was evenly spread on an MHA plate and kept for 15 minutes before being subjected to antimicrobial testing. The plates were then inverted and incubated at 35°C for 16-18 hours and the diameter of zone of inhibition was measured using a transparent ruler. The following antimicrobials with disk potencies (µg) were used (Sensi Discs, Becton, Dickinson and Company, Sparks, MD): oxacillin (Ox; 1µg), cefoxitin (Fox; 30 µg), cephalothin (Cf; 30 µg), bacitracin (B; 10 IU), clindamycin (Da; 2 µg), ampicillin (Amp; 10 µg), vancomycin (E-test; 0.016-256 mg/ml), ceftriaxone (CRO; 30 µg), chloramphenicol (Caf; 30 µg), ciprofloxacin (CIP; 5 µg), erythromycin (E; 15 µg), gentamicin (Gm; 10 µg), sulphamethoxazole-trimethoprim (Sxt; 25 µg), linezolid (Lin, 30 µg), tetracycline (Te; 30 µg), and azithromycin (Azi; 15 µg).[35,38] The methicillin susceptibility status of the isolates was based on their susceptibility to cefoxitin surrogate test.[17,27]

E-test (oxoid), a predefined, preformed, stable gradient of antibiotic concentration on a plastic strip; was used to assess isolates' susceptibility to vancomycin. Isolates with a minimum inhibitory concentration (MIC) of <2 µg/ml were considered susceptible, those with >2 µg/ml to 16 µg/ml were intermediate, and isolates with MIC>16 µg/ml were considered resistant.[14,17,28] MDR was defined as resistance to at least three classes of antimicrobials.[17,28]

Biofilm formation and strength detection

Tissue culture plate (TCP) method was used, 10 ml of TSB with 1% glucose was inoculated with a loopful of test organisms from overnight culture on nutrient agar. The broth was then incubated at 37°C for 24 hours and the culture was further diluted to 1:100 with fresh medium. Individual wells of sterile 96 wells of flat bottom TCPs were then filled with 0.2 ml of diluted cultures and a single well was inoculated with a sterile broth and served as a blank. Control organisms were also diluted and incubated similarly.[9,17,29] All three controls and blanks in the TCPs were incubated at 37°C for 24 hours. Gentle tapping of the plates was performed after incubation. Wells were washed using 0.2 ml of phosphate buffer saline (pH 7.2) four times to remove free-floating bacteria. Biofilms remained adhered to the walls and the bottoms of the wells were fixed with 2% sodium acetate and stained with 0.1% crystal violet. Extra stain was washed using deionized water and the plates were dried properly. Optical densities (OD) of stained adherent biofilm were read with a spectrophotometer at 600 nm. All experiments were run in triplicates. The average OD values of the sterile medium were calculated and subtracted from all test values.[9,14,30] OD cutoff value (ODc) was calculated by summing the average OD of the negative control + 3x standard deviation (SD) of the negative control. According to ODc and average OD, isolates were described as strong biofilm producers (4ODc≤OD); moderate biofilm producers (2ODc≤OD≤4ODc); weak biofilm producers (ODc≤OD≤2ODc); and no biofilm producers (OD≤ODc).

Data analysis

Data were compiled in Excel and analyzed using IBM SPSS version 22. Descriptive statistics summarized the data, while Chi-square tests and binary logistic regression assessed antimicrobial resistance. One-way ANOVA compared biofilm formation, with significance set at $p \leq 0.05$.

Ethical considerations

Ethical approval was granted by the institutional review board of Addis Ababa University (protocol number 033/19/SoP), and research permission to conduct the

research was also obtained from ARH. Participants received study details in their native language.

RESULTS

Sociodemographic and clinical characteristics

The mean age of participants was 30.4 years (SD=16.98), ranging from 0.06 to 80 years, with 54% in the 19-39 age group. The sex distribution was nearly equal, with males at 50.7% and females at 49.3%. A history of antimicrobial use was reported by 40% of participants, 36% had indwelling medical devices, and 33.5% had comorbidities. Regarding hospital admission, 8.4% were not admitted, 38% were hospitalized for 1-7 days, and 25.8% for more than 8 days. Healthcare professionals accounted for 27.9%, while 72.1% were patients (Table 1).

Table 1: Sociodemographic and clinical characteristics of the study participants in Tigray hospitals, north Ethiopia.

Characteristics	Number (%) of participants	Health status/unit attended							
		Patients				Medical staff			
Ward/unit attended		**MW**	**OPD**	**PW**	**SW**	**MW**	**OPD**	**PW**	**SW**
Age ranges									
<18	96 (22.3)	1	9	80	6	0	0	0	0
19-39	211 (49.07)	13	11	0	90	14	56	2	25
40-59	95 (22.1)	26	12	0	34	7	10	0	6
>60	28 (6.5)	13	2	0	13	0		0	0
Total	430	53	34	80	143	21	66	2	31
History of antimicrobial use	Y; 168 (39.06)	28	20	38	82	0	0	0	0
	N; 262 (60.93)	25	14	42	61	21	66	2	31
Total	430	53	34	80	143	21	66	2	31
Use of indwelling medical devices	Y; 155 (36.04)	33	6	40	76	0	0	0	0
	N; 275 (63.95)	20	28	40	67	21	66	2	31
Total	430	53	34	80	143	21	66	2	31
Presence of comorbidities	Y;144 (33.48)	37	14	35	58	0	0	0	0
	N;286 (66.51)	16	20	45	85	21	66	2	31
Total	430	53	34	80	143	21	66	2	31
Length of hospitalization (days)									
0	36 (8.37)	1	29	4	2	21	66	2	31
1-7	163 (37.9)	44	4	41	74	0	0	0	0
>8	111 (25.81)	8	1	35	77	0	0	0	0
Total	430	53	34	80	143	21	66	2	31
Sex									
Male	218 (50.69)	37	18	31	72	12	31	0	17
Female	212 (49.3)	16	16	49	71	9	35	2	14
Total	430	53	34	80	143	21	66	2	31

Y; Yes, and indicates the presence of an antimicrobial resistance factor; N; No, indicates its absence; MW- Medical ward; OPD- Outpatient department; PW- Pediatric ward; SW- Surgical ward, History of antimicrobial use- Patients who had taken antimicrobial for at least one treatment course depending on the drug's clinical length of treatment course.

Staphylococcus aureus carriage status and other clinical characteristics of patients

Out of 430 specimens cultured, 118 (27.4%) tested positive for *S. aureus*. The distribution by specimen type revealed that pus accounted for 52.5%, nasal swabs for 35.6%, and blood for 11.9%. *S. aureus* was identified in 34 (28.8%) medical staff and 84 (71.2%) patients. Among patients, 51 (60.7%) had a history of antimicrobial use, 31 (36.9%) had comorbidities, and 34 (40.5%) used indwelling medical devices. Of the isolates, 58 (50%) were MRSA. Significant differences were observed in MRSA carriage based on sex (p=0.005), antimicrobial use (p=0.02), hospital stay (p=0.01), and age (p<0.001). Additionally, sex-related differences in *S. aureus* and MRSA carriage among medical staff were significant (p=0.023 and p=0.009, respectively) (Table 2). The overall MRSA rate was 11.8% among medical staff and 64.3% among patients (Table 2).

Table 2: Staphylococcus aureus and MRSA carriage rates and clinical characteristics of participants in Ayder referral hospital in Tigray, northern Ethiopia.

Variables		MW	SW	PW	OPD	Total (%)	χ^2 (p value)	MRSA carriage					χ^2 (p value)
		Yes/No	Yes/No	Yes/No	Yes/No			MW	OPD	PW	SW	Total (%)	
Patients Carriage status (*S. aureus* positive)													
Age category (years)													
<18		1/0	2/0	21/	7/0	31 (36.9)	5.201 (0.023)	0	4	15	2	21 (25)	27.062 (0.000)
19-39		4/ 9	21/ 0	41	9/ 5	8 (9.52)		0	0	4	1	5 (6)	
40-59		8/ 32	20/73	5/ 13	10/14	31 (36.9)		3	2	0	12	17 (20.2)	
>60		5/34	6 /41	0/0	3/10	14 (14.3)		5	2	0	4	11 (10.7)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Type of specimen													
Nasal-swab		0/ 0	4/9	0/ 0	4/3	8 (9.52)	16.057 (0.000)	0	2	1	2	5 (5.95)	0.070 (0.791)
Blood		5/34	1/19	11/32	1/10	18 (21.43)		1	1	10	2	14 (16.7)	
Pus		5/9	24 /86	15/22	14/2	58 (69.04)		7	5	8	15	35 (46.4)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Comorbidity	Yes	6/31	6 /52	12/23	7/7	31 (36.9)	0.772 (0.38)	5	2	11	2	20 (23.8)	2.33 (0.127)
	No	4/12	23/62	14/31	12/8	53 (63.1)		3	6	8	17	34 (40.5)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Sex													
Male	158	5/32	8/64	5/26	6/12	34 (40.47)	1.217 (0.270)	5	3	5	8	22 (26.2)	8.042 (0.005)
Female	152	5/11	11/50	21/28	13/3	50 (59.53)		3	5	14	11	32 (38.1)	
Total		10/43	19/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
History of antibiotic use	Yes	7/21	20/62	14/24	10/10	51 (60.71)	11.131 (0.001)	6	6	10	15	37 (44.05)	5.405 (0.020)
	No	3/22	9/52	12/30	9/5	33 (39.29)		2	2	9	4	17 (20.2)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Indwelling medical devices	Yes	5/28	7/69	14/26	5 /1	31 (36.9)	0.101 (0.75)	5	4	12	5	26 (30.95)	0.090 (0.765)
	No	5 /15	22/45	12/28	14/14	53 (63.1)		3	4	7	14	28 (33.3)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Length of hospital stay	No	1/0	2/0	4/0	16/15	23 (27.38)	9.344 (0.025)	0	6	4	2	12 (14.3)	11.351 (0.010)
	1-7	6/37	14/60	8/41	0/0	28 (33.3)		6	2	7	6	21 (25)	
	>8	3/6	13/54	14/13	3/0	33 (39.29)		2	0	8	11	21 (25)	
Total		10/43	29/114	26/54	19/15	84		8	8	19	19	54/84 (64.3)	
Medical staff													
Male	60	4/8	4/ 13	0/0	8/23	16 (47.06)	5.201 (0.023)	0	1	0	0	1 (25)	6.923 (0.009)
Female	60	4/4	5/ 9	2/0	7/28	18 (52.94)		0	0	1	2	3 (75)	
Total	120	8/12	9/22	2/0	15/51	34		0	1	1	2	4/34 (11.76)	

*n, number of specimens; Yes; the presence of a factor; No, absence of a factor.

Antimicrobial resistance profile of S. aureus

A higher proportion of isolates (84; 71.2%) exhibited resistance to ampicillin, while only 3 (2.54%) were resistant to linezolid. Significantly higher resistance was noted in patients admitted to the surgical ward (29; 34.52%) compared to the medical ward (10; 11.9%) (p=0.000). Among medical staff, resistance was highest in the surgical ward (15; 44.12%) and lowest in the pediatric ward (2; 5.88%) (p=0.000) (Table 3, Figure 1).

Table 3: Antimicrobial susceptibility profiles of *S. aureus* isolate from participants with various factors in Ayder referral hospital, northern Ethiopia.

Factors of AMR	Amp	Tet	Azi	E	Da	Stx	Fox	Lin	Van	Caf	Gm	Ci	Ba	Oxa	Stm	Cf	Cro
Stay (0)	47	25	11	21	14	14	19	1	4	6	12	16	13	19	11	18	10
Stay (1-7)	19	20	10	12	11	10	19	1	2	6	5	13	10	19	9	18	8
Stay (>8)	18	17	9	13	11	8	20	1	3	7	8	9	13	20	8	20	5
P value	0.046	0.002	0.011	0.029	0.011	0.038	0.044	0.059	0.05	0.01	0.036	0.02	0.002	0	0.022	0	0.04
Age <18	32	24	13	21	14	15	12	1	4	6	10	17	19	12	10	28	8
Age (19-39)	40	26	14	20	19	12	26	2	3	10	11	17	12	26	12	18	12
Age (40-59)	12	10	3	5	2	6	15	0	3	4	15	5	5	15	6	8	3
>60	1	3	1	1	1	0	5	0	0	0	0	0	1	5	1	3	0
P value	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Blood	13	10	9	10	3	8	18	1	5	4	8	12	8	8	8	12	7
Nasal-swab	35	14	6	13	10	7	10	1	2	3	6	7	5	5	6	5	5
Pus	37	39	16	24	23	18	30	1	3	13	12	20	24	24	15	40	11
P value	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IMD (No)	62	42	18	30	24	18	18	2	6	9	16	22	10	18	17	30	11
IMD (Yes)	23	21	13	17	12	15	40	1	4	11	10	17	27	40	12	27	12
P value	0.129	0.087	0.033	0.063	0.126	0.01	0.011	0.156	0.12	0	0.081	0.01	0	0.01	0.042	0	0.005
HAM (No)	50	30	11	22	17	14	26	2	4	7	10	20	11	26	11	21	6
HAM (Yes)	35	33	20	25	19	19	32	1	6	13	16	19	26	32	18	36	17
P value	0.629	0.04	0.009	0.101	0.212	0.075	0.014	0.653	0.34	0.05	0.053	0.421	0	0.01	0.03	0	0.002
Health professional	27	9	4	10	7	6	4	1	2	2	4	6	4	4	5	4	3
Patient	58	54	27	37	29	27	54	2	8	18	22	33	33	54	24	53	20
P value	0.501	0.001	0.069	0.321	0.312	0.267	0.028	0.952	0.78	0.12	0.217	0.071	0.013	0.03	0.269	0	0.165
Comor (No)	65	46	18	31	25	22	19	2	6	12	17	26	22	19	19	37	19
Comor (yes)	20	17	13	16	11	11	39	1	4	8	9	13	15	39	10	20	4
P value	0.073	0.086	0.007	0.025	0.069	0.049	0.023	0.089	0.06	0.03	0.049	0.041	0.006	0.02	0.046	0.01	0.062
MW	16	10	4	6	4	7	8	1	3	4	2	4	5	8	7	7	5
OPD	20	12	4	10	9	9	7	0	1	5	7	6	6	7	5	9	2
PW	19	18	12	16	11	10	16	2	4	6	9	15	15	16	8	21	9
SW	30	23	11	15	12	7	25	0	2	5	8	14	11	25	9	20	7
P value	0.693	0.588	0.16	0.231	0.537	0.289	0.057	0.275	0.37	0.68	0.341	0.07	0.056	0.06	0.6	0.02	0.184
BF(0)	31	9	0	5	2	3	3	0	0	1	1	6	1	3	2	2	0
BF(L)	5	3	0	1	2	1	7	0	1	0	2	3	1	7	2	1	1
BF(M)	15	12	2	7	5	3	12	0	0	0	0	2	6	12	2	12	1
BF(H)	34	39	29	34	27	26	36	3	9	19	23	28	29	36	23	42	21
P value	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0

*ST; Susceptibility test, Na-s; Nasal swab, R; resistant, S; sensitive, Amp; Ampicillin, Te; Tetracycline, Azi; Azithromycin, E; Erythromycine, Da; Clindamycine, STX; Sulfa+trimethoprim, Fox; Cefoxitin, Lin; Linezolid, Van; Vancomycin, Caf; Chloramphenicol, Gm; Gentamicin, Cip; Ciprofloxacin, Ba; Bacitracin, Ox; Oxacillin, Stm; Streptomycin, Cf; Cephalothin, Cro; Ceftriaxone.

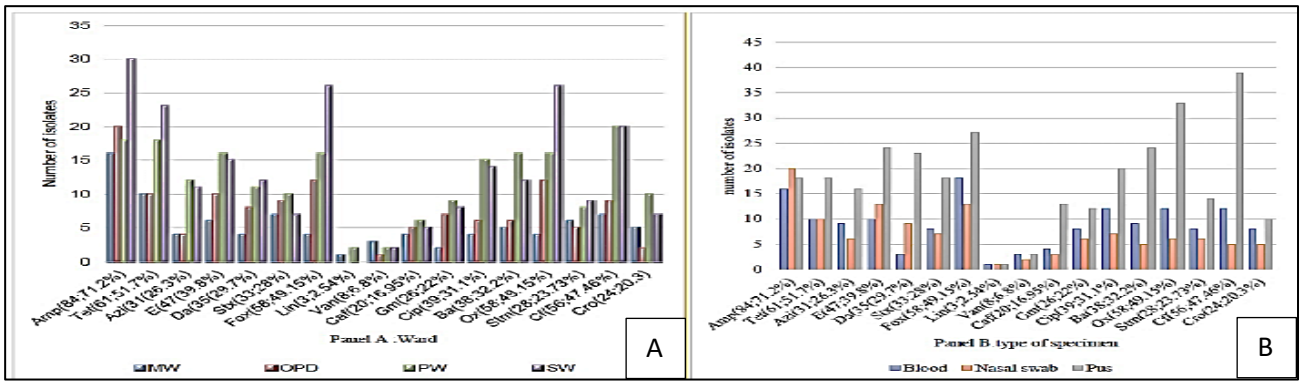

Figure 1 (A and B): Overall antimicrobial resistance patterns of S. aureus isolate from various wards and specimen types in Ayder referral hospital, Tigray, north Ethiopia.

*MW; medical ward, OPD; Outpatient department, PW; pediatric ward, SW; surgical ward, Amp; Ampicillin, Tet; Tetracycline, Azi; Azithromycin, E; Erythromycin, Da; Clindamycin, STX; Sulfamethoxazole trimethoprim, Fox; Cefoxitin, Lin; Linezolid, Van; Vancomycin, Caf; Chloramphenicol, Gm; Gentamicin, Cip; Ciprofloxacin, Ba; Bacitracin, Ox; Oxacillin, Stm; Streptomycin, Cf; Cephalotin, Cro; Ceftriaxone. Panel-A: Association of each antimicrobial resistance in different wards, Panel-B: Association of each antimicrobial resistance in different specimen types.

Table 4: Distribution of multi-drug resistance among *S. aureus* isolates against tested antibiotics in Ayder referral hospital,Tigray, north Ethiopia.

Susceptibility profile	Number of isolates (%)	Number of drugs where resistance is observed	MARI
0	32 (27.1)	0	0
Amp	22 (18.6)	1-2	0.059
Amp, Tet	6 (5.1)		0.11
Amp, Tet, Fox, Ox, Cf	10 (8.4)	>3	0.29
Amp, Tet, E, Fox, Cf	8 (6.8)		0.29
Amp, Tet, E, Fox, Cip, Ox, Cf	3 (2.5)		0.41
Amp, Tet, E, Da, Fox, Cip, Ba, Ox, Cf	3 (2.5)		0.53
Amp, Tet, E, Da, Stx, Fox, Cip, Ba, Ox, Cf	2 (1.7)		0.58
Amp, Tet, Azi, E, Da, stx, Fox, Cip, Ba, Ox, Cf	3 (2.5)		0.64
Amp, Tet, Azi, E, Da, stx, Fox, Cip, Ba, Ox, Stm, Cf	2 (1.7)		0.71
Amp, Tet, Azi, E, Da, stx, Fox, Gm, Cip, Ba, Ox, Stm, Cf	2 (1.7)		0.76
Amp, Tet, Azi, E, Da, stx, Fox, Gm, Cip, Ba, Ox, Stm, Cf, Cro	4 (3.4)		0.82
Amp, Tet, Azi, E, Da, stx, Fox, Caf, Gm, Cip, Ba, Ox, Stm, Cf, Cro	11 (9.3)		0.88
Amp, Tet, Azi, E, Da, stx, Fox, Van, Caf, Gm, Cip, Ba, Ox, Stm, Cf, Cro	7 (5.9)		0.94
Amp, Tet, Azi, E, Da, stx, Fox, Lin, Van, Caf, Gm, Cip, Ba, Ox, Stm, Cf, Cro	3 (2.5)		1.00

*MDR; multiple drug resistance (where 3 and above drugs are found to be resisted), MARI; multiple antibiotic resistance index (ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism was tested). Multiple antibiotic index (MAI>0.2) means that the high-risk source of contamination is where antibiotics are frequently used.

Table 5: Correlation between antimicrobial resistance factors and biofilm formation strength.

Factor/*S. aureus*	Strength of biofilm formation with optical density (OD)					Chi-square (p value)
	0 (None)	**Low**		**Moderate**	**High**	
Ward						
MW	7	2 (0.100-0.116)		3 (0.124-0.131)	5 (0.241-0.380)	24.024 (0.000)
OPD	19	2 (0.100-0.116)		5 (0.122-0.123)	9 (0.241-0.380)	
PW	5	1 (0.100-0.116)		4 (0.124-0.133)	18 (0.241-0.380)	
SW	10	2 (0.100-0.116)		10 (0.122-0.32)	17 (0.241-0.380)	
Total	40	7		22	49	
Age range						
≤18	9	0		7 (0.122-0.131)	21 (0.241-0380)	38.406 (0.000)
19-39	16	4 (0.100-0.116)		8 (0.122-0.131)	16 (0.241-0.380)	
40-59	15	1 (0.100-0.116)		6 (0.122-0.131)	12 (0.241-0.380)	
≥60	0	2 (0.100-0.116)		1 (0.122-0.131)	0	
Total	40	7		22	49	
Co-morbidities	No (87)	32	6 (0.100-0.116)	18 (0.122-0.131)	31 (0.241-0.380)	5.37 (0.147)
	Yes (31)	8	1 (0.100-0.116)	4 (0.122-0.131)	18 (0.241-0.380)	
Total	118	40	7	22	49	
Specimen type	Blood (14)	1	1 (0.100-0.116)	1 (0.122-0.131)	11 (0.241-0.380)	11.557 (0.073)
	Pus, (64)	17	1 (0.100-0.116)	12 (0.122-0.131)	34 (0.241-0.380)	
	Nasal-swab (40)	22	5	9 (0.122-0.131)	4 (0.241-0.380)	
Total	118	40	7	22	49	
HAM	No (67)	32	5 (0.100-0.116)	15 (0.122-0.131)	15 (0.241-0.380)	8.325 (0.04)
	Yes (51)	8	2 (0.100-0.116)	7 (0.122-0.131)	34 (0.241-0.272)	
Total	118	40	7	22	49	
IMD	No (84)	*36*	6 (0.100-0.116)	17 (0.122-0.131)	25 (0.241-0.272)	2.23 (0.526)
	Yes (34)	4	1 (0.100-0.116)	5 (0.122-0.131)	24 (0.241-0.380)	
Total	118	40	7	22	49	
Sex	Male (52)	17	6 (0.100-0.116)	10 (0.122-0.131)	19 (0.241-0.380)	3.93 (0.269)
	Female (66)	23	1 (0.100-0.116)	12 (0.122-0.131)	30 (0.241-0.380)	

Continued.

Factor/*S. aureus*		Strength of biofilm formation with optical density (OD)				Chi-square (p value)
		0 (None)	Low	Moderate	High	
Total		40	7	22	49	
LOH	0	67	36	5 (0.100-0.116)	11 (0.122-0.131)	14 (0.241-0.380)	292.3 (0.000)
	1-7	31	3	2 (0.100-0.116)	7 (0.122-0.131)	21 (0.241-0.380)	
	≥8	20	1	0	4 (0.122-0.131)	14 (0.241-0.380)	
	Total	118	40	7	22	49	

*HAM; History of antimicrobial use, IMD; indwelling medical device, LOH; length of hospitalization in days, OD; Optical density; the OD value of stained adherent biofilm for S. aureus was obtained with a spectrophotometer at wavelength 620 nm. The mean optical density was = 0.3792 and the standard deviation (SD=+0.08529).MW; Medical ward, OPD; outpatient department, PW; pediatrics ward, SW; surgical ward,

Association of antimicrobial resistance with different factors

Resistance to the panel of antibiotics was significantly associated with various clinical factors, including days of hospitalization, specimen type, ward, history of antimicrobial use, comorbidities, biofilm strength, and age range (Table 3).

Multiple antibiotic resistance index (MARI) was also calculated, and 60 (50.85%) of the isolates had a MARI of <0.2, and the remaining 58 (49.15%) were with MARI>0.2 (Table 4).

Biofilm formation

Biofilm formation was assessed using OD measurements, revealing a direct relationship between OD and biofilm-forming capacity (Table 5).

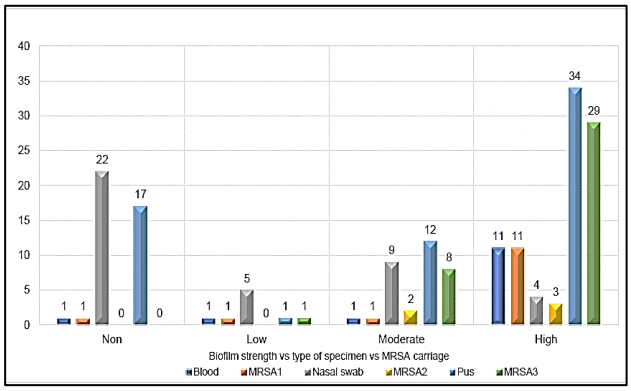

Figure 2: Biofilm strength in different types of specimens and MRSA carriage.

*Non; non-biofilm formers, Low; low biofilm formers, Moderate; Moderate biofilm formers, High; High biofilm formers, MRSA; Methicillin resistance staphylococcus aureus.

Using the TCP method, 40 isolates (33.9%) were non-biofilm-forming, while 78 isolates (66.1%) formed biofilms. Among these, 7 (8.97%) were classified as low, 22 (28.2%) as moderate, and 49 (62.8%) as high biofilm formers. High biofilm levels were particularly noted in isolates from blood (11; 78.6%), especially from the surgical ward, followed by pus (34; 72.3%) and nasal swabs (4; 22.2%) (Table 4). Notably, all blood isolates with high biofilm capacity were MRSA-positive, while 75% of nasal swab and 85.3% of pus isolates were also MRSA-positive (Figure 2).

DISCUSSION

The overall recovery rate of *S. aureus* was 27.1% among patients with various infections and 28.1% among medical staff attending these patients, highlighting *S. aureus* as a predominant pathogen in this study. This prevalence is higher compared to a previous study in Ethiopia, which reported a *S. aureus* carriage rate of 21.6%.[22] Susceptibility of the isolates to the tested drugs varied among different wards and specimen types (Table 3). This variability aligns with findings from studies conducted in Addis Ababa, Ethiopia, and Tehran.[2,23,24] This heterogeneity may be attributed to factors such as direct topical application of antimicrobial agents and empirical antimicrobial use, which could have influenced bacterial growth at the infection site.[14,25] Conversely, the lack of antimicrobial-resistant *S. aureus* in nasal swabs, while detected in pus and blood, may be due to localized colonization and infections, as observed in other studies.[13,24,26]

Among the patients, 51 (60.71%) had a history of antimicrobial use, which was significantly associated with increased *S. aureus* carriage (p=0.001). This finding is consistent with results from studies conducted in Ethiopia and Vietnam.[2,27] This could be attributed to the emergence of antimicrobial resistance (AMR) and the potential for nosocomial infections to increase microbial concentrations, as reported in studies conducted in China, Nepal, and Tanzania.[26,28,29]

In the current study, the highest resistance was observed for ampicillin (71.2%), moderate resistance was seen for sulfamethoxazole-trimethoprim (28%), and the lowest resistance was noted for linezolid (2.54%). These findings differ from other studies, where penicillin resistance was reported at 88.4%, sulfamethoxazole-trimethoprim resistance at 52.32%, and no resistance to linezolid was found.[30] Another study conducted in southern Ethiopia reported higher resistance rates for chloramphenicol (73%) and lower resistance rates for amoxicillin-clavulanic acid (32%).[31]

The current study revealed high resistance rates to most antimicrobials in *S. aureus* isolates from both pediatric (26 isolates, 30.95%) and surgical (29 isolates, 34.52%) wards, as well as in medical staff, (15, 44.12%) which is significantly higher than some studies done in different parts of Ethiopia.[2,32] This difference may be attributed to variations in patient hospitalization duration, infection control practices by healthcare facilities, use of indwelling medical devices, movement of patients with drug-resistant isolates across borders, and prior exposure to antimicrobials.[32,33]

In this study, the prevalence of MDR strains and methicillin-resistant *S. aureus* (MRSA) was 49.15% and 50%, respectively. These findings contrast with a study conducted in Zaria, where MDR was reported at 68.2% and MRSA at 18.8%.[34] and it was not also in agreement with other studies.[35] The prevalence of MRSA and MDR in this study also differs from findings in southern Ethiopia, where MDR was reported at 88.1%.[35]

The highest percentage of MRSA carriage was observed in patients under 12 years (21 cases, 38.9%) and those aged 19-40 years (21 cases, 38.9%). These rates are higher compared to findings from studies conducted in northern and western parts of Ethiopia.[13,36] These variations may be attributed to factors such as geographical diversity, the specific wards where patients are admitted, the length of hospitalization within age groups, physiological differences among age groups, and variations in antimicrobial use practices.[15,36]

MRSA carriage was significantly higher among patients with a history of antimicrobial use (37 cases, 68.52%), likely due to ongoing exposure to antimicrobials. Although *S. aureus* colonization is generally higher in males, MRSA prevalence was notably higher in females (35/58, 60.34%) compared to males (23/58, 39.7%). This discrepancy may be related to higher MRSA colonization rates associated with nosocomial infections in the wards predominantly occupied by female patients.[13,36]

Overall MRSA carriage was 58 cases (49.15%), which is higher than rates reported in other studies conducted in Ethiopia and across Africa.[13,28,36] This may be due to the persistence of MRSA in biofilms and inadequate infection prevention policies in the hospital. MRSA has the potential to spread rapidly unless stringent infection prevention and control measures are implemented.[13,37]

An association between AMR and MRSA with biofilm formation strength was observed. Isolates with higher ODs, indicating stronger biofilm formation, were found in various wards and specimen types, and a higher number of MRSA were detected (Figure 3). This finding is consistent with results from other studies.[35,38] This resistance to antimicrobials may be due to restricted penetration of drugs into biofilms, reduced growth rates, and the expression of resistance genes, as indicated by other studies.[39] Structural barriers, along with the presence of persistent cells within biofilms, play a crucial role in AMR.[7,37,39]

Most isolates from patients with a history of antimicrobial use and extended hospitalization exhibited significantly higher MDR (Table 3). This increase may be attributed to biofilm formation and the acquisition of resistant isolates during the admission period, as well as exposure to antimicrobials.[8,35,40]

Our study has certain limitations. The biofilm detection method employed, the TCP assay, relies on crystal violet staining, which may lead to an overestimation of the number of adherent bacteria. To address this issue, we used additional standards to minimize potential interference and enhance the accuracy of our results.

CONCLUSION

The increasing resistance of *S. aureus* to vancomycin and linezolid, particularly in biofilm-associated infections, presents a critical challenge. Innovative treatments and strengthened infection prevention strategies are essential to combat antimicrobial resistance and improve patient outcomes.

ACKNOWLEDGEMENTS

This work was funded by Addis Ababa University and Aksum University. The authors would like to thank all for their support.

Funding: This study was funded by Addis Ababa University and Aksum University
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of Addis Ababa University (protocol number 033/19/SoP). Permissions were obtained from Mekelle University and Ayder Referral Hospital

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Cite this article as: Gebremariam T, Eguale T, Belay T, Abula T, Engidawork E. Antibiogram profile and phenotypic characteristics of clinical isolates of *Staphylococcus aureus* at Ayder Referral Hospital, Tigray, Ethiopia. Int J Res Med Sci 2024;12:4011-21