Research Article

Prevalence of Methicillin-Resistant*Staphylococcus aureus* and Multidrug-Resistant Strains from Patients Attending the Referral Hospitals of Amhara Regional State, Ethiopia

Feleke Moges [iD],[1] **Tadele Tamiru,**[2] **Azanaw Amare** [iD],[1] **Getachew Mengistu** [iD],[3] **Setegn Eshetie,**[1] **Mulat Dagnew** [iD],[1] **Tigist Feleke,**[4] **Mucheye Gizachew** [iD],[1] **and Wondwossen Abebe** [iD][1]

[1]*Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia*
[2]*Institute of Biotechnology, College of Natural and Computational Sciences, University of Gondar, Gondar, Ethiopia*
[3]*Department of Medical Microbiology, College of Health Sciences, Debre Markos University, Debre Markos, Ethiopia*
[4]*Department of Hospital Laboratory, University of Gondar Comprehensive Specialized Hospital, Gondar, Ethiopia*

Correspondence should be addressed to Wondwossen Abebe; wondisweet@gmail.com

Background. Staphylococcus aureus (*S. aureus*) causes different types of human infections and can develop resistance to many antibiotics. There is a scarcity of data on the *mecA* gene and multidrug-resistant (MDR) strain distribution of this organism in developing countries, such as Ethiopia. This study investigated the presence of *mecA* gene and MDR profile of *S. aureus* among patients attending referral hospitals of Amhara regional state. *Methods.* Of the total of 110 isolates collected from Amhara regional referral hospitals, 70 MDR isolates were further processed for isolation of *S. aureus mecA* gene. Genomic DNA was isolated using a Sigma-Aldrich genomic DNA isolation kit for Gram-positive bacteria. Amplification of *S. aureus mecA* gene was performed with the amplicon size of 533 bp. Antimicrobial susceptibility test including methicillin resistance was determined by the Kirby–Bauer disc diffusion method. *Results.* The majority of the isolates were recovered from patients aged less than 5 years (51; 36.7%) and the least number of isolates was recorded in age group greater than 60 years (6; 4.3%). Most of the isolates were from blood (61; 43.9%), followed by wounds (45; 32.4%). A high resistance rate was observed in penicillin (81; 73.6%), followed by cotrimoxazole (78; 70.9%), ceftriaxone (76; 69%), erythromycin (66; 60%), and tetracycline (65; 59.1%). Phenotypically, considering cefoxitin as a surrogate marker, 38 (34.5%) of the isolates were methicillin-resistant. The overall MDR isolates were 80 (72.7%). The PCR amplification result of the *mecA* gene was 14 (20%). *Conclusions and Recommendations.* High rates of MDR and methicillin-resistant*S. aureus* were reported. PCR amplification indicated that 20% of MRSA isolates were the *mecA* gene carriers. Large-scale studies for the detection of MDR strains of *S. aureus* including MRSA using molecular techniques should be encouraged in the Amhara region.

1. Introduction

Staphylococcus aureus (*S. aureus*) is one of the most common causes of bacterial infection in humans that causes both community- and hospital-acquired infection of the skin, urinary tract, surgical site infections, osteomyelitis, septicaemia, and endocarditis [1]. *Staphylococcus aureus* has an extraordinary ability to develop resistance to many antibiotics. This was first revealed by the acquisition of β-lactamase on "penicillinase plasmids" and the subsequent response to β-lactamase stable derivatives by acquisition of staphylococcal cassette chromosome (SCCmec) elements by methicillin-resistant *S. aureus* (MRSA) [2].

Penicillin has been used as a drug of choice for *S. aureus* as it was discovered by Fleming in the 1940s, but with the widespread use of penicillin in the 1950s, penicillin-resistant

S. aureus appeared in the hospitals [3, 4]. Penicillin-resistant *S. aureus* can produce penicillinase, which can hydrolyze the penicillin β-lactam ring, leading to resistance to penicillin. Later, scientists developed a new penicillinase-resistant semisynthetic penicillin named methicillin, which is resistant to the hydrolysis of β-lactamase [3, 5]. Therefore, in the widespread appearance of penicillin-resistant *S. aureus*, methicillin was used as a drug of choice for penicillin-resistant *S. aureus*. However, soon later MRSA strain was reported; this resistance was produced by a gene encoding the penicillin-binding protein 2a or 2′ (PBP2a or PBP2′) (*mecA*) which was integrated into the chromosomal element (SCCmec) of methicillin-sensitive *S. aureus* [6]. Available data show that the structural gene, *mecA*, is present in the resistant strains of *S. aureus*, but not in the susceptible ones [7]. This achievement has enabled the development of an alternative method for identifying methicillin-resistant *S. aureus* by detecting the *mecA* gene. Moreover, other than *macA* gene, *mecB, mecC*, and *macD* have been documented as responsible for methicillin resistance in the *Staphylococcaceae* family. The *mecB* and *mecD* genes were reported at first on the chromosome and/or on a plasmid of *Macrococcus caseolyticus*. Recently, the *mecB* gene was also documented on a plasmid of one MRSA isolated from a human patient [8].

In the present study, the polymerase chain reaction (PCR) was used to detect the methicillin resistance determinant by amplifying a 533-bp region of the *mecA* gene. The gold standard to determine MRSA genotypes is to detect conserved genes constantly found in the *mecA* gene, which is within the range of a particular chromosome in staphylococcal cassette chromosome (SCCmec) [9]. Therefore, amplification of *mecA* can be performed by using PCR, which is the gold standard for the detection of *mecA* gene [10]. No information on the distribution of the *mecA* gene on MRSA in Amhara region is available. Therefore, this study aimed at investigating the presence of *mecA* gene and multidrug-resistant (MDR) strain distribution of *S. aureus* among patients attending referral hospitals of Amhara regional state.

2. Materials and Methods

2.1. Bacterial Isolates.

A total of 139 isolates of *S. aureus* were isolated between the periods of 2017 and 2018 from Amhara region referral hospitals (University of Gondar Comprehensive Specialized Hospital, Felege Hiwot Comprehensive Specialized Hospital, Dessie Referral Hospital, and Debre Markos Referral Hospital). Detailed data of sample size determination, sampling technique, and specimen collection were found from the previous two studies [11, 12]. All isolates were clinical isolates from different specimens such as blood, urine, wounds, discharges, and body fluids. Each clinical sample was cultured on mannitol salt agar and incubated at 37°C for 24 h. Further identification of *S. aureus* isolates was performed by colony morphology, Gram stain, and standard biochemical characteristics such as catalase, coagulase, and novobiocin susceptibility tests. The ATCC 25923 of *S. aureus* was used as reference strain.

2.2. Antibiotic Susceptibility Test.

Susceptibility test was performed using the modified Kirby–Bauer disk diffusion method on Muller–Hinton agar following Clinical and Laboratory Standard Institute (CLSI) guidelines [13]. Pure colonies of freshly grown *S. aureus* suspension were prepared in 3–5 ml normal saline and turbidity was adjusted to 0.5 McFarland standards. The plates were allowed to dry for 3–5 minutes; then, discs were evenly distributed on the inoculated plate using sterile forceps and incubated at 37°C for 18–24 h. The susceptibility test for *S. aureus* was performed against erythromycin (ERY, 15 μg), penicillin (PEN, I0 IU), clindamycin (CLI, 10 μg), cotrimoxazole (SXT, 25 μg), tetracycline (TET, 30 μg), ciprofloxacin (CIP, 5 μg), chloramphenicol (CHL, 30 μg), gentamicin (GEN, 10 μg), ceftriaxone (CRO, 30 μg), and cefoxitin (FOX, 30 μg) (all from Abtek bio.Ltd UK). Multidrug resistance patterns of the isolates were determined following the criteria set by Magiorakos et al. [14]. Using CLSI guidelines, the diameter of the zone of inhibition around the disc was measured and interpreted as sensitive, intermediate, and resistant.

2.3. Extraction of DNA from S. aureus.

The clinical isolates were subcultured using nutrient agar medium and incubated for 24 h at 37°C. A single colony was taken from the previously subcultured medium and inoculated in to 10 ml Luria–Bertani (LB) broth medium, incubated at 37°C with a shaker incubator for 24 h. After 24 h incubation, genomic DNA was isolated using Sigma-Aldrich genomic DNA extraction kit for Gram-positive bacteria, and the isolation protocols were followed according to the manufacturer's instructions of Sigma-Aldrich. Finally, the extracted DNA was dissolved with Tris-EDTA buffer (10 mM Tris-Cl and 1 mM EDTA buffer), and the quality of isolated genomic DNA was confirmed by using NanoDrop and 1.5% agarose gel electrophoresis, and then it was stored at −21°C till use.

2.4. Amplification of S. aureus mecA Gene.

MRSA isolates were identified by phenotypic method, and PCR (thermocycler machine) was performed to amplify the *S. aureus mecA* gene with the amplicon size of 533 bp using primers *mecA* forward sequence 5′-AAAATCGATGGTAAAGGT TGGC-3′ and *mecA* reverse sequence 5′-AGTTCTGGA GTACCGGATTTGC-3′ described in [15].

The specific oligonucleotide primers for *mecA* genes were diluted by using nuclease-free water according to the manufacture company information to get primary concentration equal to 100 pmol. Thermal cycler and the reaction mixtures were prepared accordingly. The PCR was performed with a total volume of 25 μl containing a mixture of 2 μl of template DNA, 2.5 μl of 10x PCR buffer, 2.5 μl (10 pmol/μl) of each *mecA* gene forward and reverse primers, 0.5 μl of dNTPS (10 mM), 1.5 μl of MgCl$_2$, and 0.5 μl of Taq polymerase, and the remaining volume was filled by nuclease-free water to get a final volume of 25 μl. PCR mixture without DNA template was used as a negative control. After preparation of mixtures, the PCR program was as follows: initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 60 s, annealing at 62°C for 30 s, extension at 72°C for 35 s, and final extension at 72°C for

Table 1: Distribution of *S. aureus* isolates in different clinical samples with respect to age from four referral hospitals of Amhara region, Ethiopia (2017-2018).

Age category	Clinical samples (%)					Total
	Urine	Blood	Wound	Discharges	Body fluids	
≤5 years	2 (1.4)	33 (23.7)	12 (8.6)	2 (1.4)	2 (1.4)	51 (36.7)
6–15 years	—	7 (5.0)	5 (3.6)	1 (0.7)	—	13 (9.4)
16–30 years	9 (9.5)	15 (10.8)	15 (10.8)	2 (1.4)	1 (0.7)	42 (30.2)
31–45 years	—	2 (1.4)	6 (4.3)	5 (3.6)	1 (0.7)	14 (10.1)
46–60 years	2 (1.4)	1 (0.7)	6 (4.3)	1 (0.7)	3 (2.2)	13 (9.4)
>60 years	1 (0.7)	3 (2.2)	1 (0.7)	—	1 (0.7)	6 (4.3)
Total	14 (10.1)	61 (43.9)	45 (32.4)	11 (7.9)	8 (5.8)	139 (100)

10 min. Finally, the PCR products were stored at 4°C until analysis by agarose gel electrophoresis.

2.5. Agarose Gel Electrophoresis. Agarose gel electrophoresis was prepared with 1.5% agarose in 1x trice acetate EDTA (TAE) buffer, and 0.5 μg/mL of ethidium bromide was added and mixed. A 12 μl volume of PCR-amplified products was mixed with 3 μl loading dye and then loaded into wells of agarose gel. Electrophoresis was carried out for 90 min (70 Volts/cm^2) in 1x TAE buffer. DNA ladder (100 bp) was used to assess the PCR product size, then PCR products were visualized by UV light at 336 nm, and photographs were taken using a digital camera.

3. Results

A total of 1365 samples were cultured, and the isolation rate of *S. aureus* was 139/1365 (10.2%). Majority of the isolates were recovered from patients aged less than 5 years (51; 36.7%), followed by 16–30 years (42; 30.2%), 31–45 years (14; 10.1%), 6–15 years (13; 9.4%), and 46–60 years (13; 9.4%). The least number of isolates was recorded in age group greater than 60 years (6; 4.3%) (Table 1).

Most of the isolates were from blood (61 (43.9%)), followed by wounds (45 (32.4%)), urine (14 (10.1%)), discharges (11 (7.9%)), and body fluids (8 (5.8%)) (Table 2). Majority of the isolates were from the University of Gondar Comprehensive Specialized Hospital (53 (38.1%)), followed by Felege Hiwot Comprehensive Specialized Hospital (37 (26.6%)), Debre Markos Referral Hospital (29 (20.9%)), and Dessie Referral Hospital (20 (14.4%)) (Table 2)).

Of the total 139 isolates collected from 4 different referral hospitals in Amhara region, 110 isolates were recovered by subculturing in the central Microbiology Laboratory at the University of Gondar. All these isolates were processed further and tested for 10 different antibiotics. High resistance rate was observed for penicillin (81; 73.6%), followed by cotrimoxazole (78; 70.9%), ceftriaxone (76; 69%), erythromycin (66; 60%), and tetracycline (65; 59.1%). However, relatively low resistance rates were observed for clindamycin ($n = 24$, 21.8%), gentamicin (34; 30.9%), and cefoxitin (38; 34.5%). Phenotypically, considering cefoxitin as surrogate marker, for methicillin resistance, 38 (34.5%) of the 110 *S. aureus* isolates were cefoxitin-resistant (Table 3) and thus classified as MRSA.

Among 110 isolates tested for 10 different commonly used antibiotics, 7 isolates were sensitive to all drugs tested and 23 isolates were resistant to one or two antibiotics. However, *S. aureus* isolates resistant to 3 or more antibiotic classes were 80 (72.7%) (Table 4).

For molecular detection of methicillin-resistant genes, out of 80 (72.7%) MDR isolates, we randomly selected 70 isolates of *S. aureus* and included from all study sites. Accordingly, we considered 40 isolates (13 were cefoxitin-resistant, 3 of them were intermediate, and 24 were sensitive) from the University of Gondar Comprehensive Specialized Hospital, 14 isolates (5 were cefoxitin-resistant, 2 of them were intermediate, and 7 were sensitive) from Felege Hiwot Comprehensive Specialized Hospital, 11 isolates (4 were cefoxitin-resistant, 3 of them were intermediate, and 4 were sensitive) from Dessie Referral Hospital, and 5 isolates (4 were cefoxitin-resistant and 1 was sensitive) from Debre Markose Referral Hospital (Table 5). In all cases, the isolates taken for *mecA* gene detection were phenotypically MDR.

The PCR amplification result of the *mecA* gene was performed in all 70 clinical isolates of *S. aureus*. However, among the total of 70 isolates, *mecA* gene was detected only in 14 (20%) *S. aureus* isolates. The *mecA* gene positive in 14 isolates was phenotypically from MRSA and MSSA, but both of them were MDR (Figure 1). Although its distribution is different, *mecA* gene producing methicillin-resistant*S. aureus* was reported in all study sites.

4. Discussions

S. aureus is a main pathogenic bacterium which causes severe human health problems globally [16], and its antimicrobial resistance characteristics have made it more rebellious in the health institutions [17].

The isolation rate of *S. aureus* in the current study was 139/1365 (10.2%) which is lower than a study conducted in Ethiopia (79/94 (84.0%)) [18] and Nigeria (55/360 (15.3%)), and the occurrence of *S. aureus* was the highest in wound swabs [19], but in the present study, the highest isolates were recovered from blood sample followed by wound specimen.

Majority of the isolates were recovered from patients aged less than 5 years (51 (36.7%)), followed by 16–30 years (42 (30.2%)), while the least number of isolates was from patients greater than 60 years. This is in line with an observation from previous Ethiopian report where the rate of isolation of *S. aureus* was higher in lower age (15–24 years)

TABLE 2: Distribution of S. *aureus* isolates from four referral hospitals of Amhara region, Ethiopia (2017–2018).

Isolates	Name of referral hospitals	Clinical samples					Total
		Urine	Blood	Wound	Discharges*	Body fluids	
S. *aureus*	University of Gondar Comprehensive Specialized Hospital	2 (3.8)	14 (26.4)	29 (54.7)	2 (3.8)	6 (11.3)	53 (100)
	Felege Hiwot Comprehensive Specialized Hospital	2 (5.4)	23 (62.2)	10 (27.0)	2 (5.4)	—	37 (100)
	Dessie Referral Hospital	8 (40.0)	1 (5.0)	3 (15.0)	7 (35.0)	1 (5.0)	20 (100)
	Debre Markos Referral Hospital	2 (6.9)	23 (79.3)	3 (10.3)	—	1 (3.4)	29 (100)
	Total	14 (10.1)	61 (43.9)	45 (32.4)	11 (7.9)	8 (5.8)	139 (100)

*Eye and ear discharges.

TABLE 3: Drug resistance patterns of *S. aureus* against commonly used antibiotics from referral hospitals of Amhara region (2017-2018).

Bacterial isolates		ERY	PEN	CLI	SXT	TET	CIP	CHL	GEN	CRO	FOX
S. aureus	S	37 (33.6)	25 (22.7)	86 (78.2)	31 (28.2)	42 (38.2)	65 (59.1)	69 (62.7)	70 (63.6)	29 (26.4)	64 (58.2)
	I	7 (6.4)	4 (3.6)	—	1 (0.9)	3 (12.7)	6 (5.5)	2 (1.8)	6 (5.5)	5 (4.5)	8 (7.3)
	R	66 (60)	81 (73.6)	24 (21.8)	78 (70.9)	65 (59.1)	39 (35.5)	39 (35.5)	34 (30.9)	76 (69.1)	38 (34.5)

ERY = erythromycin; PEN = penicillin; CLI = clindamycin; SXT = cotrimoxazole; TET = tetracycline; CIP = ciprofloxacin; CHL = chloramphenicol; GEN = gentamycin; CRO = ceftriaxone; FOX = cefoxitin; S = sensitive; I = intermediate; R = resistant.

TABLE 4: Resistance profiles of 110 *S. aureus* isolates from clinical samples at the four referral hospitals of Amhara region (2017-2018).

Antibiogram pattern	Number of *S. aureus* isolates
All drug sensitive	7
CHL (not MDR)	2
TET (not MDR)	1
SXT (not MDR)	4
FOX (not MDR)	2
TET, GEN (not MDR)	1
PEN, SXT (not MDR)	1
PEN, CRO (not MDR)	1
PEN, CHL (not MDR)	1
PEN, CLI (not MDR)	1
SXT, CRO (not MDR)	1
ERY, PEN (not MDR)	1
ERY, PEN, CRO (not MDR)	2
PEN, SXT, CRO (not MDR)	2
PEN, SXT, CRO, FOX (not MDR)	1
PEN, CHL, CRO, FOX (not MDR)	1
PEN, TET, CRO, FOX (not MDR)	1
SXT, TET, CRO (MDR)	1
PEN, SXT, TET, CRO (MDR)	1
Other isolates resistant to 3 or more antibiotic classes	78
Total non-MDR isolates	30 (27.3%)
Total MDR isolates	80 (72.7%)
Total	110 (100%)

ERY = erythromycin; PEN = penicillin; CLI = clindamycin; SXT = cotrimoxazole; TET = tetracycline; CIP = ciprofloxacin; CHL = chloramphenicol; GEN = gentamycin; CRO = ceftriaxone; FOX = cefoxitin. MDR = *S. aureus* isolates resistant to 3 or more antibiotic classes.

TABLE 5: PCR results for *mecA* gene for methicillin-resistant *S. aureus* from selected isolates at different referral hospitals of Amhara region, Ethiopia.

Study sites	Total isolates subjected for PCR	*mecA*-negative	*mecA*-positive
University of Gondar Comprehensive Specialized Hospital	40 (100)	29 (72.5)	11 (27.5)
Felege Hiwot Comprehensive Specialized Hospital	14 (100)	13 (92.9)	1 (7.1)
Dessie Referral Hospital	11 (100)	10 (90.9)	1 (9.1)
Debre Markose Referral Hospital	5 (100)	4 (80.0)	1 (20.0)
Total	70 (100)	56 (80.0)	14 (20.0)

(46/210 (21.9%)) [20] and from Eritrean study where it was significantly associated with lower age, 13 to 18 years (78.6%) and <13 years old (85.0%), and lower rate of isolation was recorded in older age (≥61 years old) [21].

The most common clinical specimen for *S. aureus* isolates in the current study was blood (61 (43.9%)), followed by wounds (45 (32.4)). However, the previous study conducted in Ethiopia demonstrated that the highest rate of isolation was observed in pus (118/213 (55.4%)), followed by nasal swab (9/27 (33.3%)) [20]; in Eritrea, highest isolates (64/103, 62.1%) were obtained from pus specimens examined, followed by blood specimens (6/15 (40.0%)) [21]. The highest prevalence of *S aureus* was also observed from seminal fluid of patients (9/36 (25%)), followed by wound swabs (13/87 (15%)) while the lowest (5.4%) was found from urine in a study from Nigeria [17]. Another study conducted in Nigeria also revealed that the occurrence of *S. aureus* was highest in wound swabs, vaginal swabs, and urine [19]. The Iranian report on distribution analysis of the *S. aureus* isolates among clinical samples showed that most of the isolates (29.0%) were recovered from the pus and the lowest (1.4%) was found from cerebrospinal fluid [15]. The
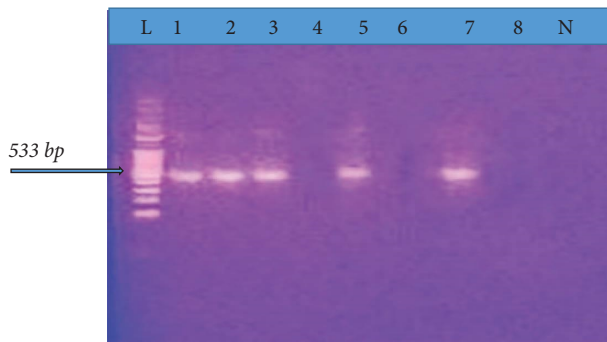
Figure 1: Agarose gel electrophoresis of the PCR amplification products of *S. aureus, mecA* gene (1.5% agarose, 70 V, 45 min.). L: the DNA molecular weight marker (100 bp ladder). Lanes 1, 2, 3, 5, and 7: positive PCR amplification of 533 bp for *mecA* gene. Lanes 4, 6, and 8: negative PCR amplification of 533 bp for *mecA* gene. N is a PCR product of negative control.

variations in occurrence of the organism in the different clinical samples across many studies show the versatility of this organism amongst other bacteria which makes it the most endemic pathogen in clinical settings, and it may likely be responsible for various infections such as UTI, wound infection, deep tissue infections, including osteomyelitis, arthritis, endocarditis, and cerebral, pulmonary, renal, and breast abscesses [22].

In the present study, the antimicrobial resistance rates of 110 *S. aureus* isolates against 10 antibiotics were 73.6%, 70.9%, 69%, 60%, 59.1%, 35.5%, 35.5%, 34.5%, 30.9%, and 21.8% toward penicillin, cotrimoxazole, ceftriaxone, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, cefoxitin, gentamicin, and clindamycin, respectively. These findings are almost in parallel with a study conducted in Ethiopia where the isolates were resistant to ampicillin (100%), cefoxitin (68.4%), clindamycin (63.3%), cephalothin (59.5%), tetracycline (57%), cotrimoxazole and bacitracin (53.2%, each), and erythromycin (51.9%) [18], and in Iran where the percentage of resistance of *S. aureus* was 100%, 59.1%, 57.7%, 50%, 49.1%, 48.3%, 47.6% and 47.6%, 25%, and 0.7% to penicillin, tetracycline, ciprofloxacin, erythromycin, gentamicin, cotrimoxazole, cephalothin, and oxacillin, clindamycin, and vancomycin, respectively [15]. The highest level of antimicrobial resistant *S. aureus* in a Nigerian study was 68% to ceftazidime, followed by cloxacillin (48%) while the least resistance (26%) was observed for meropenem [17]. In line with the current study, another study from Nigeria also demonstrated that the isolates from three hospitals were highly (≥50%) resistant to all the antibiotics tested (ampicillin, ciprofloxacin, erythromycin, oxacillin, rifampicin, clindamycin, sulphamethoxazole/trimethoprim, and streptomycin), but moderately (≤40%) resistant to gentamicin and levofloxacin [19]. This variation might be attributed to differences in patients' hospital stay, level of infection control practices by health facilities, previous exposure of patients to antibiotics, and irrational use of antibiotics.

Phenotypically, considering cefoxitin as surrogate marker for methicillin test, 38 (34.5%) of the isolates of *S. aureus* were methicillin-resistant in the current study which is in agreement with the pooled prevalence of MRSA reported in Ethiopia (32.5%) [23]. However, the current finding of MRSA is lower than a report from Ethiopia, where 54 (68.4%) of the isolates were MRSA [18]; from Eritrea, 59 (72.0%) of the isolates were MRSA [21]; from Nigerian studies, 44.0% [17]; and 40.4% of the isolates were MRSA [24]; and from Iran, 133/279 (47.6%) of the isolates were MRSA [15]. On the other hand, the present report is higher than another previous report from Ethiopia where 34/194 (17.5%) of the *S. aureus* isolates were found to be MRSA [20]; in Iraq, the prevalence of MRSA was 114/429 (26.54%) [25]. The possible explanation for the observed discrepancies across the literature might be associated with the variation of the methods used to detect methicillin resistance. Some studies used cefoxitin and others used oxacillin as a surrogate marker for the detection of methicillin resistance.

The MDR isolates observed in the current study was 80/110 (72.7%) which is in line with a previous report in Ethiopia (65 (82.3%)) [18]. However, the MDR *S. aureus* observed in the present study is higher than a previous study reported from Ethiopia where 98 (50.5%) of the *S. aureus* isolates were MDR [20], from Eritrea where 17/43 (39.5%) isolates were MDR [21]; and from Saudi Arabia where 47% of MRSA were MDR [26].

The PCR amplification result of *mecA* gene, a gene that confers resistance to methicillin and most β-lactam antibiotics, was obtained in 70 clinical isolates of *S. aureus*. However, among the total of 70 isolates, *mecA* gene was detected only in 14 (20.0%) *S. aureus* isolates with an amplicon of 533 bp considered as indicative with the presence of *mecA* gene. Although its distribution is different, *mecA* gene producing MRSA was reported in all study sites. This is similar with a study from Nigeria that phenotypic resistance to cefoxitin was 46.5%, while the *mecA* gene was 19.2% [24]. Another study from Nigeria indicated that *S. aureus* isolates with phenotypic resistance to methicillin (oxacillin) were tested for *mecA* gene and none of the isolates contained the *mecA* gene [27]. Nwaogaraku et al. from Nigeria showed that all isolates of MRSA from blood samples of pigs were *mecA* negative on PCR [28]. However, the present study is different from many studies performed elsewhere [26, 29, 30]. The possible explanation why phenotypically MRSA-positive isolates did not show *mecA* gene might be due to loss of the *mecA* gene during prolonged storage [31] or other mechanisms other than the presence of *mecA* gene (*mecC* and *mecB*) responsible for methicillin-resistant *Staphylococcus aureus* [32, 33].

5. Conclusion and Recommendation

Phenotypic resistance to cefoxitin was 34.5%. This prevalence overestimated the prevalence of MRSA, as the *mecA* gene that encodes resistance to methicillin was detected by PCR in 20.0% of the *S. aureus* isolates. A large-scale study for *mecA* gene detection is important to re-assure the

discrepancy between phenotypic and *mecA* gene detection in methicillin-resistant S. *aureus.*

Data Availability

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board of the University of Gondar with reference number O/VIP/RCS/05/478/2015.

Consent

Informed written consent was obtained from each study participant. Children less than 18 years who are not able to give consent were also asked an assent and/or written consent taken from their parents or guardians.

Disclosure

A preprint has previously been published by Moges et al. in Research Square on 16 February 2022 [34].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

FM was involved in conception of the research idea, study design, data collection, analysis and interpretation, and the drafting of the manuscript; TT and AA performed laboratory work; GM performed data analysis; SE contributed to study design and data analysis and interpretation; MD was involved in data analysis and write up; TF and MG performed data collection and laboratory work; WA contributed to conception of the research idea, study design, data analysis and interpretation, and write up. All authors read and approved the final manuscript.

References

[1] D. Guillemot, S. Bonacorsi, J. S. Blanchard et al., "Amoxicillin-clavulanate therapy increases childhood nasal colonization by methicillin-susceptible Staphylococcus aureus strains producing high levels of penicillinase," *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 12, pp. 4618–4623, 2004.

[2] T. J. Foster, "Antibiotic resistance in Staphylococcus aureus. Current status and future prospects," *FEMS Microbiology Reviews*, vol. 41, no. 3, pp. 430–449, 2017.

[3] C. Rayner and W. J. Munckhof, "Antibiotics currently used in the treatment of infections caused by Staphylococcus aureus," *Internal Medicine Journal*, vol. 35, no. s2, pp. S3–S16, 2005.

[4] S. Pichereau and W. E. Rose, "Invasive community-associated MRSA infections:epidemiology and antimicrobial management," *Expert Opinion on Pharmacotherapy*, vol. 11, no. 18, pp. 3009–3025, 2010.

[5] S. Khoshnood, M. Heidary, A. Asadi et al., "A review on mechanism of action, resistance, synergism, and clinical implications of mupirocin against Staphylococcus aureus," *Biomedicine & Pharmacotherapy*, vol. 109, pp. 1809–1818, 2019.

[6] R. H. Schulte and E. Munson, "Staphylococcus aureus resistance patterns in Wisconsin: 2018 surveillance of Wisconsin organisms for trends in antimicrobial resistance and epidemiology (SWOTARE) program report," *Clinical Medicine and Research*, vol. 17, no. 3-4, pp. 72–81, 2019.

[7] M. D. Song, S. Maesaki, M. Wachi et al., "Primary structure and origin of the gene encoding the β- lactam-inducible penicillin-binding protein responsible for methicillin resistance in Staphylococcus aureus," in *Antibiotic Inhibition of Bacterial Cell Surface Assembly and Function*, P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman, Eds., pp. 352–359, American Society for Microbiology, Washington, DC, USA, 1988.

[8] C. Ngassam Tchamba, J. N. Duprez, P. Lucas et al., "Comparison of the staphylococcal chromosome cassette (SCC) mec in methicillin-resistant Staphylococcus aureus (MRSA) and non-aureus staphylococci (MRNAS) from animals and humans," *Antibiotics*, vol. 10, no. 3, p. 256, 2021.

[9] A. Jain, A. Agarwal, and R. K. Verma, "Cefoxitin disc diffusion test for detection of meticillin-resistant staphylococci," *Journal of Medical Microbiology*, vol. 57, no. 8, pp. 957–961, 2008.

[10] D. Jonas, M. Speck, F. D. Daschner, and H. Grundmann, "Rapid PCR based identification of methicillin-resistant Staphylococcus aureus from screening swabs," *Journal of Clinical Microbiology*, vol. 40, no. 5, pp. 1821–1823, 2002.

[11] F. Moges, M. Gizachew, M. Dagnew et al., "Multidrug resistance and extended-spectrum beta-lactamase producing Gram-negative bacteria from three Referral Hospitals of Amhara region, Ethiopia," *Annals of Clinical Microbiology and Antimicrobials*, vol. 20, no. 1, pp. 16–22, 2021.

[12] F. Moges, S. Eshetie, W. Abebe et al., "High prevalence of extended-spectrum beta-lactamase-producinggram-negative pathogens from patients attending Felege Hiwot comprehensive specialized hospital, bahir dar, Amhara region," *PLoS One*, vol. 14, no. 4, 2019.

[13] Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*, CLSI, 27th edition, Wayne, PA, USA, 2017.

[14] A. P. Magiorakos, A. Srinivasan, R. Carey et al., "Multidrug resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance," *Clinical Microbiology and Infection*, vol. 18, no. 3, pp. 268–281, 2012.

[15] A. Pournajaf, A. Ardebili, L. Goudarzi, M. Khodabandeh, T. Narimani, and H. Abbaszadeh, "PCR based identification of methicillin-resistant Staphylococcus aureus strains and

their antibiotic resistance profiles," *Asian Pacific Journal of Tropical Biomedicine*, vol. 4, no. Suppl 1, pp. S293–S297, 2014.

[16] M. B. Jan, J. D. Turnidge, and A. Sentry, "High prevalence of Oxacillin-resistant S. aureus isolates for hospitalized patients in Asia-Pacific and South Africa. The sentry antimicrobial surveillance program, 1998-1999," *Journal of Antimicrobial Chemotherapy*, vol. 46, no. 3, pp. 879–887, 2002.

[17] E. E. Ibadin, I. O. Enabulele, and F. Muinah, "Prevalence of mecA gene among staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria," *African Health Sciences*, vol. 17, no. 4, pp. 1000–1010, 2017.

[18] S. Tadesse, H. Alemayehu, A. Tenna et al., "Antimicrobial resistance profile of Staphylococcus aureus isolated from patients with infection at tikur anbessa specialized hospital, addis ababa, Ethiopia," *BMC Pharmacol Toxicol*, vol. 19, no. 1, p. 24, 2018.

[19] O. D. Angel, P. S. Kadarko, J. S. Muazu et al., "Antimicrobial resistance profile and molecular detection of MecA gene in methicillin resistant Staphylococcus aureus from patients in selected general hospitals in Abuja municipal, Nigeria," *GSC Biological and Pharmaceutical Sciences*, vol. 7, no. 3, pp. 093–106, 2019.

[20] T. Dilnessa and A. Bitew, "Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus isolated from clinical samples at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia," *BMC Infectious Diseases*, vol. 16, no. 1, p. 398, 2016.

[21] E. Y. Garoy, Y. B. Gebreab, O. O. Achila et al., "Methicillin-resistant Staphylococcus aureus (MRSA): prevalence and antimicrobial sensitivity pattern among patients-A multi-center study in asmara, Eritrea," *Eritrea. Can J Infect Dis Med Microbiol.,*vol. 2019, Article ID 8321834, 9 pages, 2019.

[22] S. Y. Daniyan, M. Galadima, I. Ujj, and M. Oda, "In vitro susceptibility profile of Methicillin-resistant Staphylococcus aureus isolates from clinical specimens to commonly used Antibiotics in Minna, Nigeria," *Asian Journal of Pharmaceutical and Health Sciences*, vol. 1, no. 3, pp. 128-129, 2011.

[23] S. Eshetie, F. Tarekegn, F. Moges, A. Amsalu, W. Birhan, and K. Huruy, "Methicillin resistant Staphylococcus aureus in Ethiopia: a meta-analysis," *BMC Infectious Diseases*, vol. 16, no. 1, p. 689, 2016.

[24] O. A. Olowe, O. O. Kukoyi, S. S. Taiwo et al., "Phenotypic and molecular characteristics of methicillin-resistant Staphylococcus aureus isolates from Ekiti State, Nigeria," *Infection and Drug Resistance*, vol. 6, pp. 87–92, 2013.

[25] A. H. Al-Marzoqi, H. Azize, T. Al-Dulaimi, and A. Nada, "Phenotypic detection of resistance in Staphylococcus aureus isolates: detection of (mec A and fem A) gene in methicillin-resistant Staphylococcus aureus (MRSA) by polymerase chain reaction," *Journal of Natural Sciences Research*, vol. 4, no. 2, pp. 112–118, 2014.

[26] A. Albarrag, A. Shami, A. Almutairi, S. Alsudairi, S. Aldakeel, and A. Al-Amodi, "Prevalence and molecular genetics of methicillin-resistant Staphylococcus aureus colonization in nursing homes in Saudi Arabia," *The Canadian Journal of Infectious Diseases & Medical Microbiology*, vol. 2020, Article ID 2434350, 6 pages, 2020.

[27] B. O. Olayinka, A. T. Olayinka, A. F. Obajuluwa, J. A. Onaolapo, and P. F. Olurinola, "Absence of mecA gene in methicillin-resistant Staphylococcus aureus isolates," *African Journal of Infectious Diseases*, vol. 3, no. 2, pp. 49–56, 2010.

[28] C. N. Nwaogaraku, S. I. Smith, and J. A. Badaki, "Non detection of mecA gene in methicillin resistant Staphylococcus

[29] aureus isolates from pigs," *African Journal of Clinical and Experimental Microbiology*, vol. 20, no. 2, pp. 159–163, 2019.

[29] S. J. M. Siyahkali, O. Dadras, Z. Ali, S. Seyedalinaghi, and N. Hejazi, "Evaluation of sensitivity and specificity of the method cefoxitin-disk diffusion in detection of methicillin-resistant Staphylococcus aureus in clinical samples of two hospitals, tehran, Iran," *Journal of International Translational Medicine*, vol. 6, no. 3, 2018.

[30] V. Velasco, E. Buyukcangaz, J. S. Sherwood, R. M. Stepan, R. J. Koslofsky, and C. M. Logue, "Characterization of Staphylococcus aureus from humans and a comparison with isolates of animal origin, in north Dakota, United States," *PLoS One*, vol. 10, no. 10, 2015.

[31] A. van Griethuysen, I. van Loo, A. van Belkum et al., "Loss of the mecA gene during storage of methicillin-resistant Staphylococcus aureus strains," *Journal of Clinical Microbiology*, vol. 43, no. 3, pp. 1361–1365, 2005.

[32] C. Milheiriço, H. de Lencastre, and A. Tomasz, "Full-genome sequencing identifies in the genetic background several determinants that modulate the resistance phenotype in methicillin-resistant Staphylococcus aureus strains carrying the novel mecC gene," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 3, pp. e02500–e02516, 2017.

[33] K. Hiramatsu, T. Ito, S. Tsubakishita et al., "Genomic basis for methicillin resistance in Staphylococcus aureus," *Infection & Chemotherapy*, vol. 45, no. 2, pp. 117–136, 2013.

[34] F. Moges, T. Tamiru, A. Amare et al., *mecA-Gene Detection and MDR Profile of S. aureus Isolates from Patients Attending the Referral Hospitals of Amhara Reginal State, Ethiopia*, Research Square, Durham, North, Carolina, 2021.