

mecA-Gene Detection and MDR Profile of *S. Aureus* Isolates From Patients Attending The Referral Hospitals of Amhara Regional State, Ethiopia

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Research

Keywords: *S. aureus*, Multidrug resistance, Methicillin resistant *S. aureus*, *mecA* gene

Posted Date: November 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1013602/v1>

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Abstract

Background: *Staphylococcus aureus* causes different types of human infections, and has an ability to develop resistance to many antibiotics. There is scarcity of data on *mecA* gene and MDR profiles of this organism in developing countries, like Ethiopia. The aim of the present study is therefore, to investigate MDR profiles and *mecA*-gene profile of *S. aureus* from Referral Hospitals of Amhara Regional State.

Methods: Of the total of 110 isolates collected from Amhara Region Referral Hospitals, 70 MDR isolates were further processed for isolation of *S. aureus mecA* gene. Genomic DNA was isolated using SIGMA ALDRICH genomic DNA isolation Kit for Gram positive bacteria. Amplification of *S. aureus mecA* gene was done using amplicon size of 533 bp. Agarose gel electrophoresis was prepared with 1.5% agarose by TAE solvent and 0.5µg/mL of ethidium bromide. Electrophoresis was carried out for 1 hour and a half (7 Volts/ cm²) in 1X tris acetate EDTA (TAE) buffer. Data were analysed by using descriptive statistics.

Results: Majority of the isolates were recovered from patients age less than 5 years, 51 (36.7%) and least from age greater than 60 years, 6 (4.3%). Most of the isolates were from blood, 61 (43.9%), followed by wound, 45 (32.4%). 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 ceftiofloxacin as a surrogate marker, 38 (34.5%) of the isolates were methicillin resistant. The overall MDR isolates were 92 (83.6%). The PCR amplification result of *mecA* gene was 14 (20%).

Conclusions and recommendations: There are high rate of MDR and MRSA isolates of *S. aureus*. PCR amplification result indicates 20% of MRSA isolates are *mecA* gene producers. Large scale study for detection of MDR strains of *S. aureus* including MRSA using molecular techniques should be encouraged in Amhara region.

Introduction

Staphylococcus aureus is one of the most common causes of bacterial infection in human 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 to which it has been exposed. 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 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 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.

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 (fixed/ preserved) constantly found in *mecA* gene, which is within the range of a particular chromosome in Staphylococcal Cassette Chromosome (SCC*mec*) (8). Therefore, amplification of *mecA* can be done by using polymerase chain reaction (PCR), which is the gold standard for the detection of *mecA* gene (9). No information on the distribution of *mecA* gene on MRSA in Amhara Region is available. Therefore, a study on this gene is important.

Materials And Methods

Bacterial isolates

A total of 139 isolates of *S. aureus* were isolated between the periods of 2017-2018 from Amhara Region Referral Hospitals (University of Gondar Comprehensive Specialized Hospital, Felege Hiwot Comprehensive Specialized Hospital, Dessie Referral Hospital, Debre Markos Referral Hospital). All isolates were clinical isolates from different specimens such as blood, urine, wound, discharges and body fluids. Each clinical sample was cultured on mannitol salt agar and incubated at 37⁰C for 24 hrs. Further identification of *S. aureus* isolates was done by colony morphology, Gram stain, standard biochemical characteristics such as catalase, coagulase and novobiocin susceptibility tests. The ATCC 25923 of *S. aureus* was used as reference strain.

Antibiotic susceptibility testing

Susceptibility test was done using modified Kirby-Bauer disk diffusion method on Muller-Hinton agar following Clinical and Laboratory Standard Institute (CLSI) guide lines (10). Pure colonies of freshly grown *S. aureus* suspension was prepared with equivalent 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 testing for *S. aureus* was done against erythromycin (ERY, 15 µg), penicillin (PEN, 10 IU), clindamycin (CLI, 10 µg), cotrimoxazole (SXT, 25µg), tetracycline (TET, 30µg), ciprofloxacin (CIP, 5µg), chloramphenicol (CHL, 30µg), gentamycin (GEN, 10µg), ceftriaxone (CRO, 30µg) and ceftioxin (FOX, 30µg) (all from Abtek bio.Ltd UK). Multi-drug resistance patterns of the isolates were determined following the criteria set by Magiorakos et.al. (11). Using CLSI guide lines, the diameter of the zone of inhibition around the disc was measured and interpreted as Sensitive, Intermediate, and Resistance.

Extraction of DNA from *S. aureus*

The clinical isolates were sub cultured using nutrient agar medium and incubated for 24hr at 37⁰c. A single colony was taken from the previously sub cultured medium and inoculated in to 10ml Luria-Bertani (LB) broth medium, incubated at 37⁰c with a shaker incubator for 24hrs. After 24hrs incubation genomic DNA was isolated using SIGMA ALDRICH genomic DNA extraction Kit for Gram positive bacteria and the isolation protocols were according to manufacturer's instructions of SIGMA ALDRICH. Finally, the extracted DNA was

dissolved with Tris-EDTA buffer (TE), the quality of isolated genomic DNA was confirmed by using nano drop and 1.5% agarose gel electrophoresis and then stored at -21⁰c till used.

Amplification of *S. aureus mecA* gene

Seventy 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'-AAAATCGATGGTAAAGGTTGGC-3' and *mecA* reverse primer sequence 5'-AGTTCTGGAGTACCGGATTTGC-3' described by (12).

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 reaction was contained a total volume of 25µl with a mixture of 2µl of template DNA, 2.5µl of 10x PCR buffer, 2.5 µl (10 pmol/µl) of each *mecA* gene forward and reverse primers, 0.05µl of dNTPS (10mM), 1.5 µl of MgCl₂, 0.05µl of Taq polymerase and the remaining volume was filled by nuclease free water to get final volume of 25µl. PCR mixture without DNA template was used as a negative control. After preparation of mixtures, the PCR program was done for 30 cycles to amplify 533bp of *mecA* gene with a subsequent step; initial denaturation at 94⁰C for 5 min; denaturation at 94⁰C for 60sec; annealing at 62⁰C for 30sec; extension at 72⁰C for 35sec and final extension at 72⁰C for 10 min. Finally, the PCR product was held at 4⁰c until it was analysed by agarose gel electrophoresis.

Agarose Gel Electrophoresis

Agarose gel electrophoresis was prepared with 1.5% agarose by TAE solvent and 0.5µg/mL of ethidium bromide was added and mixed. PCR amplified products of *S. aureus mecA* gene 533 bp (12µl) mixed with 3 µl loading dye then loaded into wells of agarose gel. Electrophoresis was carried out for 1 hour and a half (70 Volts/ cm²) in 1X trice acetate EDTA (TAE) buffer. DNA ladder (100bp) was used to assess PCR product size, then PCR products were visualized by UV light at 336 nm, and photographs were taken using digital camera.

Results

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

Table-1. Distribution of *S. aureus* isolates in different clinical samples with respect to age from four Referral Hospitals, Amhara Region, Ethiopia; 2017-18.

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

Most of the isolates were from blood, 61 (43.9%), followed by wound, 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 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).

Table-2. Distribution of *S. aureus* isolate from four Referral Hospitals, Amhara Region, Ethiopia; 2017-18.

Isolates	Name of Referral Hospitals	Clinical samples					Total
		Urine	Blood	Wound	Discharges*	Body fluids	
<i>S. aureus</i>	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							

Of the total 139 isolates collected from 4 different Referral Hospitals in Amhara region, 110 isolates were recovered by sub-culturing 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 in penicillin 81 (73.6%) followed by cotrimoxazole 78 (70.9%), ceftriaxone 76 (69%), erythromycin 66 (60%) and tetracycline 65 (59.1%). However, clindamycin 24 (21.8%), gentamycin 34 (30.9%) and Cefoxitin 38 (34.5%) were relatively with low resistance rate. Phenotypically, considering cefoxitin as surrogate marker, for methicillin resistance, 38 (34.5%) of the isolates of *S. aureus* were resistance for cefoxitin (Table-3).

Table-3. Drug resistance patterns of *S. aureus* against commonly used antibiotics from Referral Hospitals of Amhara Region; 2017-18

Bacterial Isolates		ERY	PEN	CLI	SXT	TET	CIP	CHL	GEN	CRO	FOX
<i>S. aureus</i>	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)
Total		110 (100)	110 (100)	110 (100)	110 (100)	110 (100)	110 (100)	110 (100)	110 (100)	110 (100)	110 (100)
ERY= Erythromycin; PEN= Penicillin; CLI= Clindamycin; SXT = Cotrimoxazole; TET = Tetracycline; CIP = Ciprofloxacin; CHL = Chloramphenicol; GEN = Gentamycin; CRO = Ceftriaxone; FOX= Cefoxitin.											

Among 110 isolates tested for 10 different commonly used antibiotics, 7 isolates were sensitive to all drugs tested and 16 isolates were resistant to one or two antibiotics. However, *S. aureus* isolates resistant to 3 or more antibiotic classes were 87 (79.1%) (Table-4).

Table-4. Multidrug resistance profile of *S. aureus* from clinical samples at the four Referral Hospitals, Amhara Region; 2017-18

Antibiogram pattern	Number of <i>S. aureus</i> 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 (MDR)	1
SXT, TET, CRO (MDR)	1
Other isolates resistant to 3 or more antibiotic classes	85
Total Non MDR isolates	23 (20.9%)
Total MDR-isolates	87 (79.1%)
Total	110 (100%)
ERY= Erythromycin; PEN= Penicillin; CLI= Clindamycin; SXT = Cotrimoxazole; TET = Tetracycline; CIP = Ciprofloxacin; CHL = Chloramphenicol; GEN = Gentamycin; CRO = Ceftriaxone; FOX= Cefoxitin. MDR = <i>S. aureus</i> isolates resistant to 3 or more antibiotic classes.	

For molecular detection of methicillin resistant genes, we selected MDR isolates of *S. aureus* and include from all study sites. Accordingly, we considered 40 isolates from University of Gondar Comprehensive Specialized Hospital, 14 isolates from Felege Hiwot Comprehensive Specialized Hospital, 11 isolates from Dessie Referral Hospital and 5 isolates 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 *mecA* gene was done 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 with an amplicon of 533 bp considered as indicative with the presence of *mecA* gene (Fig. 1). Although, its distribution is different *mecA* gene producing methicillin resistant *S. aureus* were reported in all study sites.

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	<i>mecA</i> -negative	<i>mecA</i> -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)

Discussions

S. aureus is a main pathogenic bacterium which causes severe human health problems globally (13), and its anti-microbial resistance characteristics has made it more rebellious in the health institutions (14).

The isolation rates 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%) (15); Nigeria, 55/360 (15.3%), and the occurrence of *S. aureus* was the highest in wound swabs (16), but in the present study, the highest isolates was recovered from blood sample followed by wound specimen.

Majority of the isolates were recovered from patients age less than 5 years, 51(36.7%), followed by 16-30 years, 42 (30.2%) while the least number of isolates were 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), 46/210(21.9 %) (17), and in 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) (18).

The most common clinical specimen for *S. aureus* isolates in the current study was blood 61 (43.9%) followed by wound, 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%) (17); in Eritrea, highest isolates (64/103, 62.1%) were obtained from pus specimens examined followed by blood specimens 6/15 (40.0%) (18). 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 urine samples showed the least (5.4%) in a study from Nigeria (14). Another study conducted in Nigeria also revealed that the occurrence of *S. aureus* was highest in wound swabs, high vaginal swabs and urine (16). The Iranian report on distribution analysis of the *S. aureus* isolates among clinical samples showed that most isolates (29.0%) were recovered from the pus and the lowest (1.4%) was found from cerebrospinal fluid (12). The variations in occurrence of the organism in the different clinical samples across many studies shows 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 infection such as UTI,

wound infection, deep tissue infections, including osteomyelitis, arthritis, endocarditis, and cerebral, pulmonary, renal and breast abscesses (19).

In the present study, the resistant rate of *S. aureus* isolates against 10 antibiotics were, 81(73.6%), 78(70.9%), 76(69%), 66(60%), 65(59.1%), 39(35.5%) and 39(35.5%), 38 (34.5%), 34(30.9%), and 24(21.8%) to penicillin, cotrimoxazole, ceftriaxone, erythromycin, tetracycline, chloramphenicol and ciprofloxacin, ceftazidime, gentamicin and clindamycin, respectively; which is almost in parallel with a study conducted in Ethiopia where the isolates were resistant to ampicillin (100%), ceftazidime (68.4%), clindamycin (63.3%), cephalothin (59.5%), tetracycline (57%), cotrimoxazole and bacitracin (53.2%, each), and erythromycin (51.9%) (15); and in Iran where the percentage of resistance of *S. aureus* were to 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, co-trimoxazole, ceftazidime and oxacillin, clindamycin and vancomycin, respectively (12). 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 (14). In line with the current study, another study from Nigeria also demonstrated that the isolates from three hospitals were highly ($\geq 50\%$) resistant to all the antibiotics tested (Ampicillin, Ciprofloxacin, Erythromycin, Oxacillin, Rifampicin, Clindamycin, Sulphamethoxazole/Trimethoprim, and Streptomycin), but moderately ($\leq 40\%$) to gentamicin and levofloxacin (16). 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, irrational use of antibiotics.

Phenotypically, considering ceftazidime 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% (20). However, the current finding of MRSA is lower than a report from Ethiopia, where 54 (68.4%) of the isolates were MRSA (15); from Eritrea, 59(72.0%) of the isolates were MRSA (18); from Nigerian studies, 44.0% (14); and 40.4% (21); and from Iran 133/279 (47.6%) (12). 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 (17); and Iraq, MRSA prevalence was 114/429 (26.54%) (22). 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 ceftazidime and others used oxacillin as a surrogate marker for detection of methicillin resistance.

The MDR isolates observed in the current study was 87/110 (79.1%) which is in line with a previous report in Ethiopia, 65(82.3%) (15). 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* were MDR (17); in Eritrea, 17/43(39.5%) (18); and in Saudi Arabia where 47% of MRSA were MDR (23).

The PCR amplification result of *mecA* gene, a gene that confers resistance to methicillin and most β -lactam antibiotics, was done 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 were reported in all study sites. This is similar with a study from Nigeria that, phenotypic resistance to ceftazidime was 46.5%, while the *mecA* gene was 19.2% (21). 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 (24). As reported by Nwaogaraku et al from Nigeria showed that, all isolates of MRSA from blood samples of pigs were *mecA* negative on PCR (25). However, the present study is different from many studies done elsewhere (23, 26, 27). 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 (28) or other mechanisms other than the presence of *mecA* gene (*mecC* and *mecB*) responsible for methicillin resistant *Staphylococcus aureus* (29, 30).

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*.

List Of Abbreviations

MDR: Multidrug resistance; MRSA: Methicillin resistant *S. aureus*; CLSI: Clinical and Laboratory Standard Institute.

Declarations

Acknowledgements

The authors would like to thank Fierhiwot Mulugeta, Tigist Birku, Senaiyt Tadesse, Tsehaynesh G/eyesus Aynalem Mohammed, and Abebaw Bitew for their laboratory work and we also acknowledge study participants.

Authors' Contributions

FM: conception of the research idea, study design, data collection, analysis and interpretation, and the drafting of the manuscript; TT: Laboratory work, AA: Laboratory work; GM: Data analysis; WA: Data analysis and write up; SE: study design, data analysis and interpretation; MD: Data analysis and write up; TF: Data collection and laboratory work; MG: Data collection and laboratory work. All authors read and approved the final manuscript.

Funding: No external funds obtained only institutional support by the University of Gondar (VP/RCS/05/192/2015), Ethiopia.

Availability of data and materials

All data generated or analysed during this study were included in this article.

Ethical approval and consent to participate: Ethical approval was obtained from the institutional review board of the University of Gondar with reference number O/VIP/RCS/05/478/2015. Informed written consent was obtained from each study participants. 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.

Competing interests

The authors declare that they have no competing interests.

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Figures

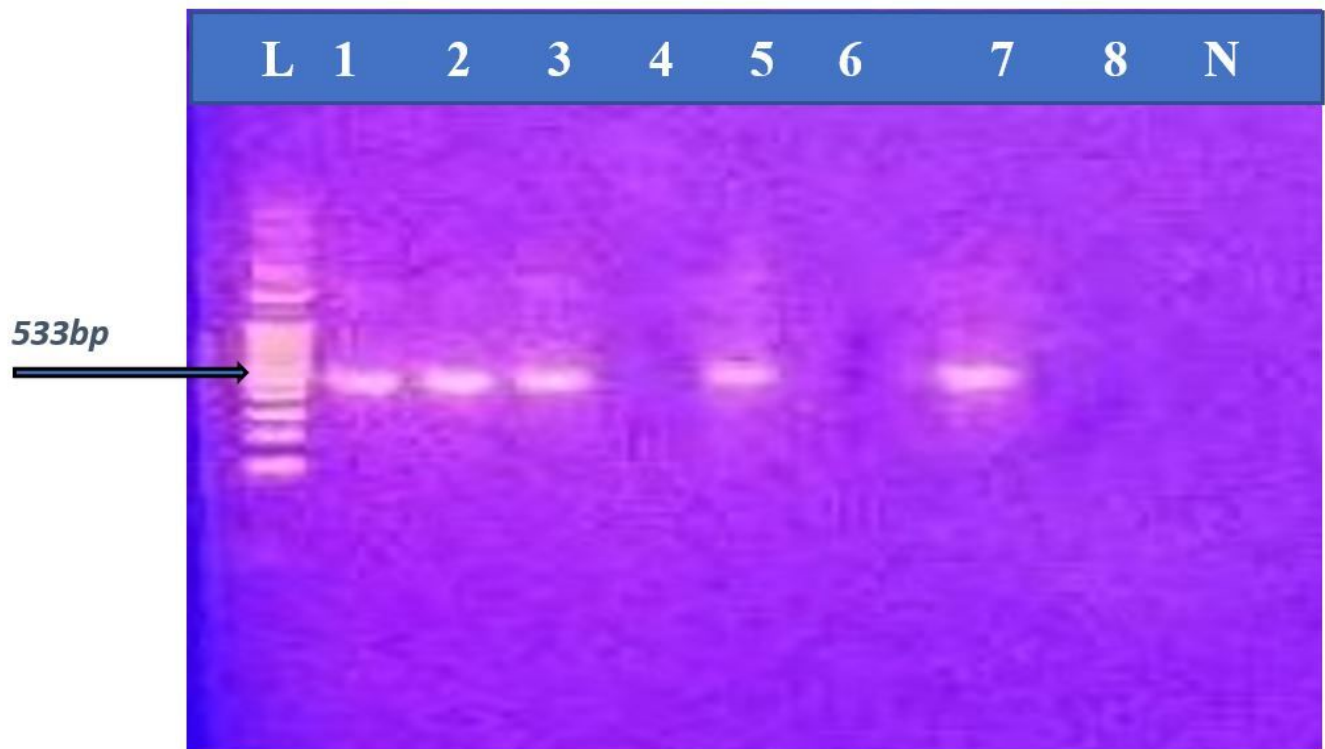


Figure 1

Agarose gel electrophoresis of PCR amplification products of *S. aureus*, *mecA* gene (1.5% agarose, 70V, 45min.). L: The DNA molecular weight marker (100pb ladder). Lanes (1, 2,3,5, and 7) positive PCR amplification of 533 bp for *mecA* gene. Whereas 4, 6, and 8 Negative PCR amplification of 533 bp for *mecA* gene. N is a PCR product of negative control.