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Original Research Article

The Association of MECA Gene Polymorphism and Drug Resistance Pattern of Methicillin-Resistant *Staphylococcus Aureus* Isolated from *Keha* and *Shinta* Rivers of Gondar Town, Northwest Ethiopia

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ABSTRACT

Associated with nosocomial and community-acquired infections, Staphylococcus aureus is a potentially hazardous human bacterium that is alarmingly developing drug resistance. The current study's objective was to assess the association of the mecA gene polymorphism and drug resistance pattern of Methicillin-resistant Staphylococcus aureus isolated from Keha and Shinta rivers of Gondar town, Northwest Ethiopia. A purposive sampling technique was used to collect 10 water samples from different sites of the two rivers. The bacterial isolates were identified using standard morphological and biochemical methods, followed by susceptibility testing to known antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) was detected by using the standard PCR method using a specific pair of primers. Genomic DNA of the isolates was isolated using the DNA Extraction Kit (GenElute, USA). Amplification of the mecA gene was done by PCR using specific primers for the mecA gene. The PCR products were visualized using agarose gel electrophoresis with a 1.5% gel. The results indicated that four (66.7%) Methicillin-Resistant Staphylococcus aureus isolates showed have 499 bp band size of the mecA gene. S. aureus showed a wide range of resistance, with the highest levels observed for ampicillin (100%), penicillin, chloramphenicol, erythromycin, and tetracycline (66.7%). Instead, S. aureus had variable sensitivity to gentamycin (100%) and ciprofloxacin (100%) as well as vancomycin (66.7%). On the other hand, 66.7 %) bacterial isolates showed resistance to several antibiotics. The result of this finding was that S. aureus isolates with the mecA gene were more resistant to many antibiotics than mecA-negative isolates. In addition, the present study confirmed that the treated wastewaters mixed with the two rivers are potential sources of S. aureus and Methicillin-Resistant Staphylococcus aureus infections. This might be due to the poor wastewater treatment methods followed by several wastewater-generating point sources in the study site. In conclusion, our findings suggest that the Keha and Shinta Rivers in Gondar Town may be potential receptacles for MRSA, which is capable of infecting both exposed humans and animals.

Keywords: Antibiotic resistance, Molecular detection, Urban River, Gene polymorphism.

INTRODUCTION

The bacterium *Staphylococcus* was first identified in 1880 at Aberdeen, Scotland, in pus from a surgical abscess (Puma *et al*., 2017). *Staphylococcus aureus* is a Gram-positive *coccal bacterium*, which may or may not contain a capsule of polysaccharides. They are characterized as non-motile and with no spore-forming facultative anaerobes that produce enzymes like catalase and coagulase (Akanbi *et al*., 2017).

S. aureus is a likely fatal human pathogen that is linked to nosocomial and community-acquired disease and is alarmingly developing antibiotic resistance (El-Rahman *et al*., 2021). Earlier studies on *S. aureus* in rivers have associated bathers with the spread of the bacteria in river water (Khan, 2016), since they expel it from their noses, skin, and respiratory tracts. It has also been confirmed to be prevalent on recreational beaches' water and sand, directly correlated with swimmers' density and nearby human activities (Akanbi *et al*., 2017; Thapaliya *et al.*, 2017; Water & Organization, 2006). Every year, biological contamination of urban rivers is predicted to be accountable for millions of gastrointestinal and acute respiratory diseases, in addition to skin infections (Das *et al*., 2017; Hashemi *et al*., 2018).

Misuse of antibiotics results in an increase in antibiotic resistance, treatment failure, and leads to the spread of antibiotic-resistant infectious microbes in environmental samples (Liu *et al.*, 2016; Román *et al*., 2025; Tamhankar & Stålsby Lundborg, 2019). Comparably, studies have shown that hospital wastewater is highly responsible for releasing high levels of resistant bacteria into the natural environment (Galler *et al*., 2018). As confirmed by Brooks *et al* (Brooks *et al*., 2014) confirmed the existence of bacteriophages from animal fecal wastes as an environmental vector for the horizontal transfer of antibiotic resistance genes results in highly resistant infectious bacteria.

S. aureus has been rapidly developing resistance to almost any antibiotic drug (Galler *et al.*, 2018). It has been widely reported that *S. aureus* is resistant to antimicrobial drugs, and this resistance has significantly contributed to the treatment's failure (Thapaliya *et al*., 2017). In 1961, the date marking the development of Methicillin-resistant *Staphylococcus aureus*, methicillin resistance, suggesting resistance to all beta-lactam agents, was first identified. Any *S. aureus* strain known as MRSA has developed resistance to methicillin and other beta-lactam drugs (Nasution *et al*., 2018). It is the root of numerous difficult infections in people (Khan, 2016).

The staphylococcal chromosome cassette mec (SCCmec), mobile gene element (mecA gene), which codes coding penicillin-binding protein 2a (PBP2a) in *S. aureus,* makes the microbe resistant to methicillin (Havaei *et al*., 2012; Pournajaf *et al*., 2014). This particular protein has less affinity for beta-lactam antibiotics (Rajamani *et al*., 2017). Previously, the presence of antibiotic-resistant bacteria in water was reported and is possibly due to the existence of antimicrobial residues in water and high concentrations of microorganisms that facilitate the exchange of genetic material (Porrero *et al*., 2012; Qiao *et al*., 2018; Purohit *et al*., 2017). In particular, in the river and water environments, MRSA has been reported to survive (Genetie and Abetu, 2020). Thus, their survival and life in water prompts the hope that this study will isolate MRSA from Gondar City's two most famous rivers, typically used by middle- and lower-class citizens.

In Ethiopia, most of the research has been conducted mainly on antimicrobial susceptibility approaches for the detection of MRSA, including the disk diffusion method. Unfortunately, limited research has been done on bacteriological quality assessments of urban river waters and those of several wastewater streams. Similar studies showing the incidence of MRSA in urban rivers were not previously performed in Ethiopia, including the study area, Gondar town. Earlier research reports are mainly limited to microbial characterization and identification of MRSA from clinical

samples. Thus, this research work aims to assess the association of the mecA gene polymorphism and the drug resistance pattern of MRSA isolated from the *Keha* and *Shinta* rivers of Gondar town, Northwest, Ethiopia.

MATERIALS AND METHODS

Study Area Description

The study was carried out in Gondar town, which is located in Gondar city which is located in the Northwestern Ethiopia, Amhara National Regional State. Gondar is located approximately 734 km from Addis Ababa. Geographically, Gondar is bounded by 120 35' 07'' North latitude and 370 26' 08'' East longitude with an altitude range of 2000-2200m above sea level and with 20^{o}c annual average temperature, 1172 mm average annual rainfall. The total land area of Gondar city is estimated to be about 5560 hectares with a total population of 300,000 (Abrha *et al*., 2016).

Sample Size And Sample Collection

A total of 10 urban river water samples from ten different sampling sites were intentionally chosen from the *Keha* and *Shinta* Rivers. In the former river, four samples were collected, and each sample site was designated as "Khw1 and Khw2: after the junction of hospital waste into to *Keha* river; Kb1 and Kb2: before the junction of hospital waste into the river." Meanwhile, six samples were collected from the second river and labeled as "Sb1 and Sb2: *Shinta* river before the junction of Dashen beer factory waste; Dw1 and Dw2: after the junction of the waste to *Shinta* river; Op1 and Op2: after the junction of university of Gondar oxidation pond, downstream to the junction of Dashen beer factory waste to *Shinta* river" represent where *S. aureus* organisms were isolated. "Khw1, Kb1, Dw1, Op1, and Sb1" were taken at the surface, and Khw2, Kb2, Dw2, Op2, and Sb2 were taken in some depth (50cm) at the same sampling sites of the rivers.

Water samples (100ml) were collected from each sampling site using presterilized plastic bottles and transferred to the respective laboratory in an ice box for studying their bacteriological quality assessments (Adesoji *et al*., 2019).

Bacteriological Analysis

Isolation of *S. Aureus*

This was achieved by continuous cultivation and sub-culturing using the spread plate technique by streaking the organism into the prepared media (Nutrient agar, Manitol salt agar, and later on Blood agar) using suitable aseptic-conditioned inoculation loops. Several colony characteristics, including size, shape, color, and differential characteristics, such as pigmentation, were considered for initial identification of bacterial isolates. Each unique colony was sub-cultured by quadrant streaking on nutrient agar plates to obtain a pure culture, following the previous studies' procedure with some modifications (Faridi *et al*., 2018). Colonies on blood agar and manitol salt agar that were creamy white and yellow, respectively, were picked up aseptically and subjected to additional biochemical analysis, biochemical tests like catalase and coagulase tests (CLSI, 2016; Cheesbrough, 2005).

Antimicrobial Susceptibility Testing

Susceptibility of isolates was tested to 8 antibiotics, including penicillin (10 µg), ampicillin (10 µg), vancomycin (30 µg), gentamicin (10 µg), tetracycline (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), and ciprofloxacin (5 µg) (Mast, Merseyside, UK), using agar disk diffusion method on Mueller-Hinton agar plates, as recommended by Clinical and Laboratory Standards

Institute (CLSI, 2018). Intermediate results were considered as resistant (Seyedmonir *et al*., 2015; Seid *et al*., 2023).

Genomic Dna Extraction

The genomic DNA of the isolates showing antibiotic resistance was extracted from pure culture (1.5 ml) grown overnight in Liquid broth using the DNA Extraction Kit (GenElute, USA) following the manufacturer's guidelines. The purity and concentration of isolated Genomic DNA were confirmed by means of NanoDrop (Thermo Scientific™, Nano-400, China).

Amplification And Detection Of Meca Gene Using Pcr Technique

The PCR amplification of the target antibiotic-resistant gene was performed using the DNA amplification instrument Master cycler Gradient (TECHNE, Germany). The specific primer pairs forward (5'AAAATCGATGGTAAAGGTTGGC 3') and Reverse (5' AGTTCTGGAGTACCGGATTTGC 3') were used for amplification of a 499 (bp) sized mecA gene fragment (Pournajaf *et al*., 2014). The PCR reaction mixture (20 µL) consisting 2 µL of template DNA, 4 µL of 5x Hot start Master Mix (Ampliqon, USA), including 1X PCR buffer, 1.5 mmol/L MgCl2, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA polymerase (Ampliqon Co., Denmark), 0.5 µL of 10 pm each primer, and 12.6 µL of sterile distilled water. The thermal cycling protocol for PCR amplification was comprised of pre- pre-denaturation at 94 °C for 3 min, denaturation at 94°C for 30 sec, annealing at 58°C for 30 seconds, extension at 72°C for 1 min, final extension at 72°C for 7 min, and final hold at 4°C, followed by 33 cycles. The PCR results were confirmed with gel electrophoresis using 1.5% agarose gels stained with ethidium bromide. Meanwhile, their size was determined using a 100 bp DNA ladder. Known antibiotic-resistant strains of *S. aureus* (ATCC43300) were used as a positive control (Abdulghany and Khairy, 2014).

Data Analysis

SPSS statistical package version 20 was used to analyze the experimental data to determine the frequency and percentage of the bacterial isolates' resistance and susceptibility to the available antibiotics.

RESULT

Isolation And Characterization Of Bacterial Isolates

A total of 10 samples were tested, from *Keha* River (n=4) and *Shinta* River (n=6), of which 6 isolates were confirmed as *S. aureus*, from which four isolates were MRSA by both culture and molecular characterization, and two were MSSA. Just 7 (70 %) of the 10 colonies were found to be gram-positive, and six of them were grouped in clusters (like a bunch of grapes), and one was microscopically long-rod shaped. The remaining 3 (30%) were Gram-negative, *coccal*-shaped bacteria. The grapes like bacteria 6 (60%) were later identified as *S. aureus* after biochemical test (Table 3).

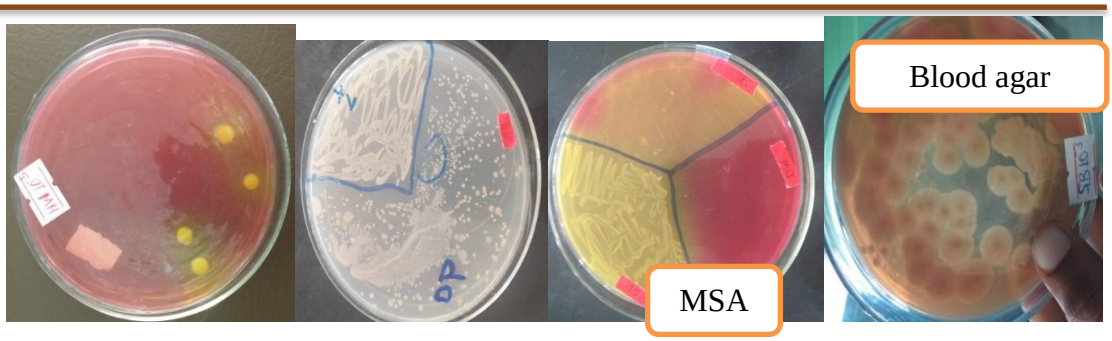


Figure 1: Morphological characterization of isolates on MSA, nutrient agar, and blood agar.

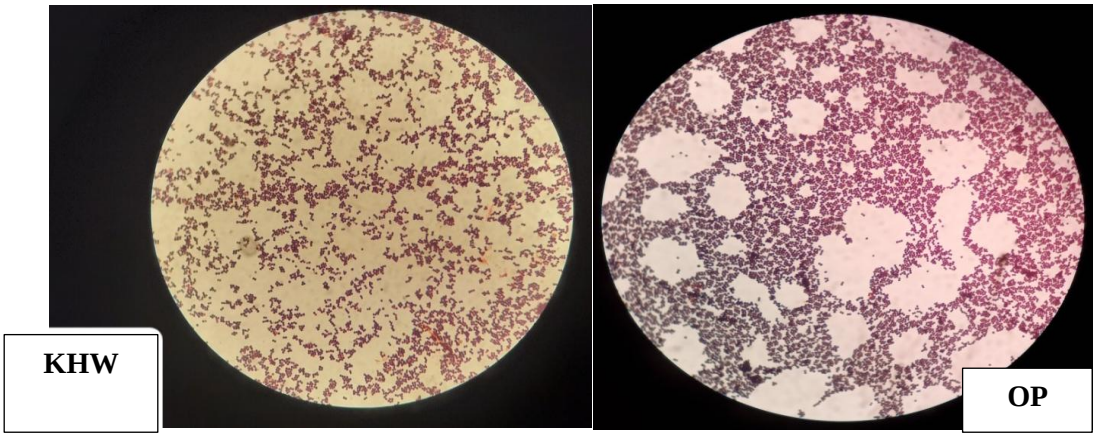


Figure 2: Microscopic view of isolates (Gram–positive) arranged in clusters (grape-like).

Table 1: Morphological characterization of isolates on Nutrient Agar.

Bacterial isolates	Colony Pigment	Bacterium shape	Colony Shape	Colony nature	Transparency
Khw1	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Khw2	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Kb1	White	Short road	Irregular	Smooth	Transparent
Kb2	White	Long Road	Circular	Smooth	Transparent
Dw 1	White	Long-Road	Circular	Smooth	Transparent
Dw 2	Yellow	Long-Road	Irregular	Smooth	Opaque
Op 1	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Op 2	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque

Sb 1	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Sb 2	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque

Table 2: Further morphological characterizations of the isolates on selective and differential media.

Isolates	Media type				
	Mannitol Salt Agar (MSA)		Blood Agar		
	Colony color	Colony shape	Colony color	Colony shape	Hemolytic activity
Khw1	Yellow	Circular	Golden	Round	beta-hemolytic
Khw2	Yellow	Circular	Golden	Round	beta-hemolytic
Kb1	Pink	Circular	non-pigmented	Irregular	non-hemolytic
Kb2	Pink	Circular	non-pigmented	Irregular	non-hemolytic
Dw 1	Red	Irregular	non-pigmented	Round	non-hemolytic
Dw 2	Red	Irregular	non-pigmented	Irregular	non-hemolytic
Op 1	Yellow	Circular	Golden	Round	beta-hemolytic
Op 2	Yellow	Circular	Golden	Round	beta-hemolytic
Sb 1	Yellow	Circular	Golden	Round	beta-hemolytic
Sb 2	Yellow	Circular	Golden	Round	beta-hemolytic

where "Khw1 and Khw2: after the junction of hospital waste into Keha river; Kb1 and Kb2: before the junction hospital waste into the river: Sb1 and Sb2: Shinta river before the junction of Dashen beer factory waste; Dw1 and Dw2: after the junction of Dashen beer factory waste to Shinta river; Op1 and Op2: after the junction of university of the Gondar oxidation pond, downstream to the junction of Dashen beer factory waste to Shinta river" represent where S. aureus organisms were isolated. Khw1, Kb1, Dw1, Op1, and Sb1 were taken at the surface, and Khw2, Kb2, Dw2, Op2, and Sb2 were taken in some depth (50cm) of the same river sampling sites.

BIOCHEMICAL TEST-BASED ISOLATION AND IDENTIFICATION OF BACTERIAL ISOLATES

Biochemical tests were subjected to isolates which were appeared as yellow and golden color colonies on mannitol salt agar (MSA) and nutrient agar (NA) media, respectively, and showed complete hemolysis of sheep red blood cells on blood agar (BA). As shown in Table 3 below, all were positive for Gram's reaction, catalase test, coagulase test, mannitol fermentation, and blood hemolysis except for isolates of Kb1, Kb2, Dw1, and Dw2. In general, isolates (Khw1, Khw2, Sb1, Sb2, Op1, and Op2) were identified as S. aureus using the biochemical test results.

Table 3: Biochemical characterizations of isolates

Isolate	Gram reaction	Catalase test	Coagulase taste	Mannitol fermentation	Blood hemolysis

Khw 1	+	+	+	+	+
Khw2	+	+	+	+	+
Kb 1	-	-	-	+	-
Kb 2	-	+	-	-	-
Dw 1	-	+	-	+	-
Dw 2	+	-	-	-	-
Op 1	+	+	+	+	+
Op 2	+	+	+	+	+
Sb 1	+	+	+	+	+
Sb 2	+	+	+	+	+

Key: (+) plus sign indicates a positive result, while (-) sign shows a negative result for isolates to grams reaction, catalase, coagulase, mannitol fermentation, and blood hemolysis test.

ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

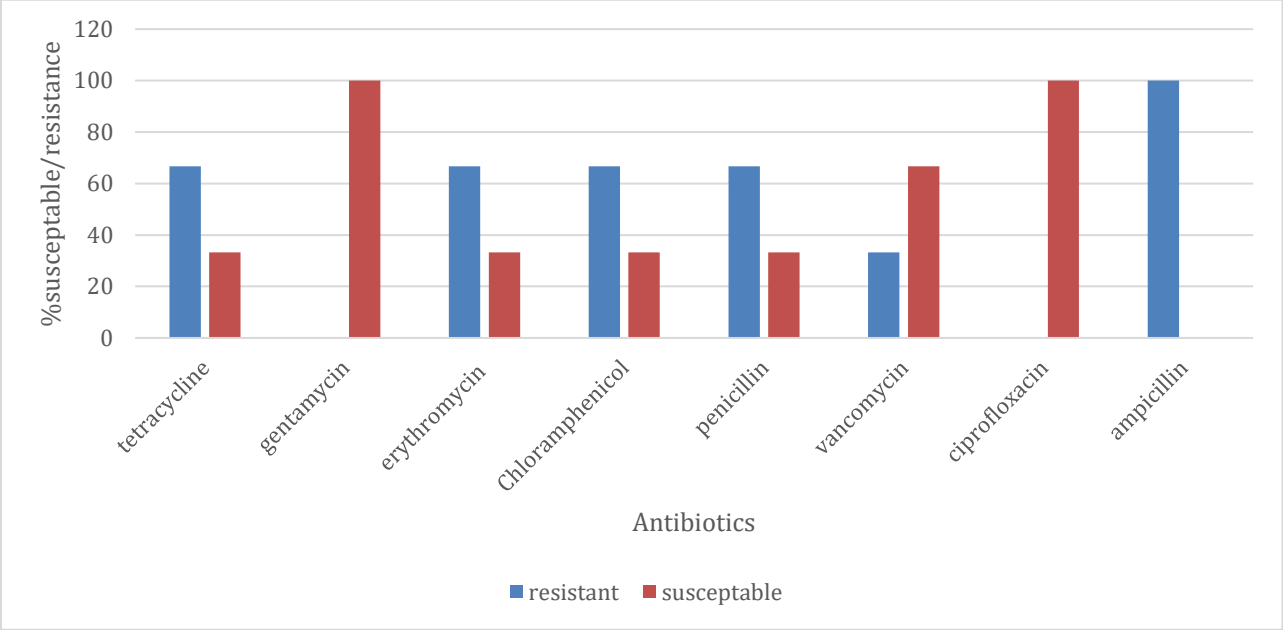
Six of the bacterial isolates showed varying susceptibility patterns to the antimicrobial agents. Generally, Ciprofloxacin 100% (6/6), Gentamycin 100% (6/6), and Vancomycin 66.7% (4/6) were the most effective antibiotics against the isolates. However, as represented in the bar chart (Figure 4), a lower susceptibility, ≤ 35% was recorded to Erythromycin, Chloramphenicol, Penicillin, and Tetracycline, 33.3% (2/6), and Ampicillin 0% (6/6). Thus, based on the multiple antibiotic resistance test, four of the six isolates (Khw1, Khw2, Op1, and Op2) were multidrug-resistant *S. aureus* and were believed to contain the mecA gene, which was later confirmed by PCR amplification.

Table 4: Antimicrobial sensitivity pattern of *S. aureus* strains to different antimicrobial agents

Isolates	Tet	Gen	Ery	Chlor	Pen	Van	Cipro	Amp
Khw 1	R	S	R	R	R	R	S	R
Khw2	R	S	R	R	R	R	S	R
Op 1	R	S	R	R	R	S	S	R
Op 2	R	S	R	R	R	S	S	R
Sb 1	S	S	S	S	S	S	S	R
Sb 2	S	S	S	S	S	S	S	R
Susceptibility (%)	2(33.3%)	6(100%)	2(33.3%)	2(33.3%)	2(33.3%)	4(66.7%)	6(100%)	0

Were, S= Susceptibility and R= Resistance

Chlor = Chloramphenicol, Van = vancomycin, Amp = ampicillin, Tet = tetracycline, Pen= penicillin, Gen = gentamycin, Ery = erythromycin and Cipro = ciprofloxacin



ISOLATION AND QUANTIFICATION OF CELLULAR DNA

The purity and concentration values of the isolated template (genomic) DNA were within the range recommended for PCR reaction, as shown in Figures 5A and B below.

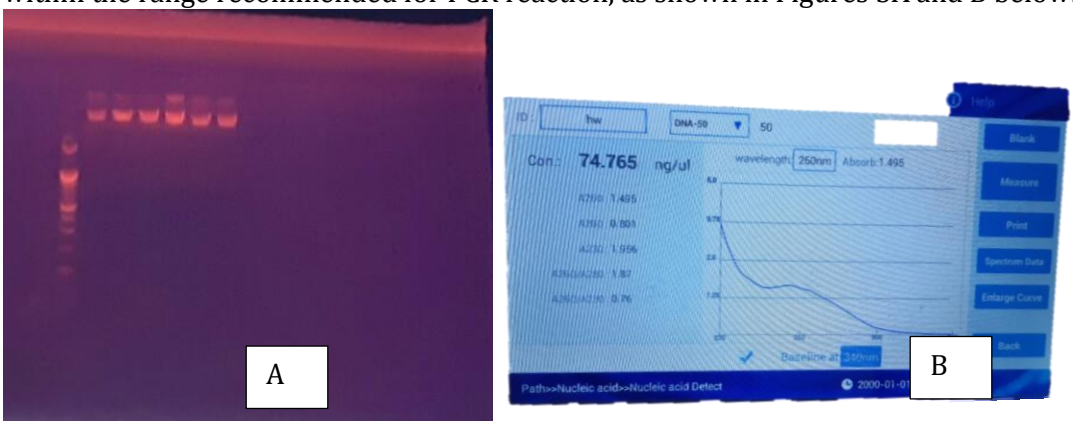


Figure 4 A and B: The view of genomic DNA and its concentration using gel electrophoresis and Nanodrop, respectively.

PCR-BASED DETECTION OF THE MECA GENE OF MRSA

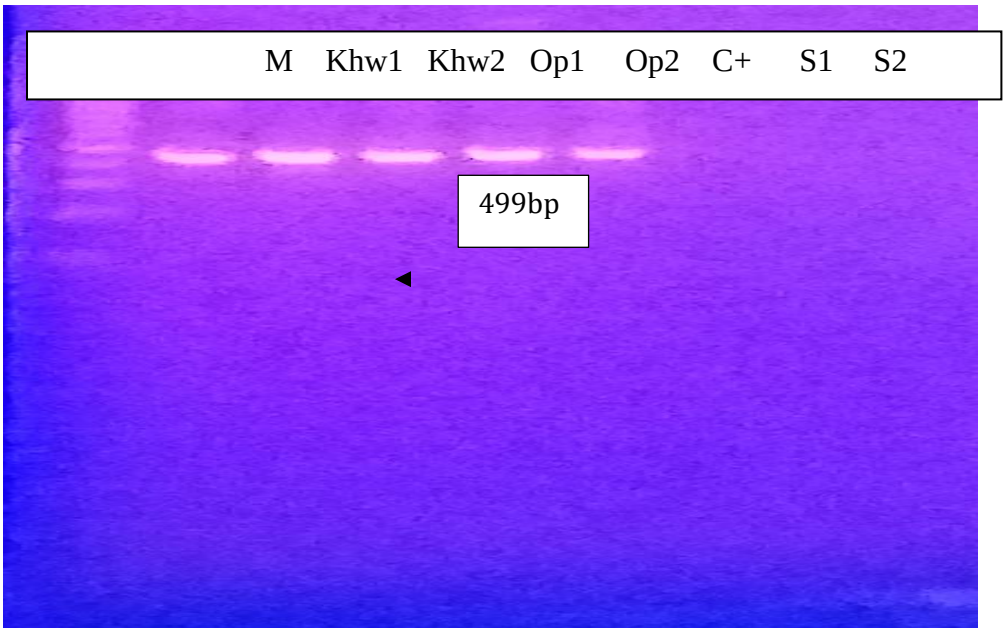


Figure 5: Represents the amplified mecA gene for pre-screened antibiotic-resistant *S. aureus* isolates. Lane S1 and S2: mecA-negative *S. aureus*; lane Khw1, Khw2, Op1, and Op2: PCR product of the mecA gene (499 bp); M: 100 bp size DNA marker; C+: Known antibiotic resistance strains of *S. aureus* (ATCC43300) (positive control), using 1.5 % agarose.

DISCUSSIONS

Poor management of hospital waste and oxidation pond sewage discharge is considered the point source of urban river pollutants and causes aquatic biodiversity and water quality degradation (Manzoor and Sharma, 2019; Cunningham and Gharipour, 2018; Mateo-Sagasta et al., 2017). Particularly, medical waste, including antibiotics, pathogens, and their products, is the most common components of clinical wastewater, which facilitate the development of antibiotic-resistant microbes in water environments (Wear and Thurber, 2015; Prüss-Ustün et al., 2019). Thus, we have conducted this scientific research as a useful strategy for improving human health and environmental sustainability using biotechnological techniques.

The results of this study confirmed that the prevalence of MRSA isolates (40%) is found to be relatively similar to the study conducted in Nigeria, domestic waters of Gombe city (30.7%) (Genetie and Abetu Arega, 2020). In addition, the results of this study confirmed that the prevalence of MRSA was found to be higher than the study conducted in Egypt (14.5%) (Fogarty *et al*., 2015) and in South Africa (22.7%) (Akanbi *et al*., 2017). Among the total of six *S. aureus* isolates tested in the study, four were methicillin-resistant *S. aureus* (MRSA), two from each river were identified using antibiotic susceptibility and molecular testing, and the remaining two were MSSA. This was similar to *S. aureus* and MRSA bacterium, which were isolated from the explored river water samples (Genetie and Abetu, 2020; Das *et al*., 2017). Six *S. aureus* isolates, two from the Keha River and four from the Shinta River water samples, were identified in the present study; these sites are considered primary pollutant junctions of the rivers.

There is a serious public health concern due to the increase in *S. aureus* and MRSA in the rivers, which hastens the occurrence of community-acquired diseases around the world (Gómez *et al*., 2016; Fri *et al*., 2020). Additionally, certain hospital and commercial antibiotics that are present in river water can disrupt the microbial communities (Genetie and Abetu, 2020; Akanbi, 2017), resulting in pathogens with antibiotic resistance, like this study isolate MRSA, via horizontal gene transfer (Porrero *et al*, 2012).

Most of the isolated *S. aureus'* susceptibility and resistance patterns revealed a high degree of resistance to the most tested antimicrobials, including penicillin (10 µg), ampicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), and chloramphenicol (30 µg). This finding agrees with previous research that reported the occurrence of multi-drug and methicillin-resistant *Staphylococcus aureus* (MRSA) from various sources of domestic water (Das *et al*., 2017; Genetie and Abetu, 2020; Mohammed *et al*., 2018). Of the isolates evaluated in this study, resistance of *S. aureus* to penicillin was extremely high (100%; 6/6), while resistance to tetracycline and ampicillin was 66.7% (4/6). However, our findings differ from those reported by Hatcher *et al*. (Garoy et al., 2019). All isolates, on the other hand, were susceptible to ciprofloxacin (5 µg), gentamicin (10 µg) and vancomycin (30 µg), similar to *S.aureus* isolated from patients in Asmara, Eritrea (Gómez *et al*., 2016), which is unlike to the previous study conducted by Gabriella and his colleagues' (Nasution *et al*., 2018), but it was in-line with to those of an earlier study in South Africa and northeast Ohio (Akanbi *et al*., 2017; Das *et al*., 2017). The resistance profile in this study appeared to be higher because the isolates were collected from samples of river water mixed with wastewater from hospitals, academic institutions, municipal, and domestic origins (Hatcher *et al*., 2016).

High resistance to β-lactam antibiotics was expected, given that *S. aureus* has been resistant to penicillin since the 1960s, and ampicillin is one of the most widely used antibiotics for treating infections in humans and animals (Abdulghany and Khairy, 2014). Isolates exhibiting resistance to ampicillin may concurrently develop resistance to other β-lactam antibiotics through cross-selection mechanisms (Garoy *et al*., 2019; Aziz and Hassan, 2019; Ghaznavi-Rad & Ekrami, 2018). This phenomenon was observed in our study, where ampicillin-resistant isolates also exhibited resistance to erythromycin, chloramphenicol, penicillin, and tetracycline, consistent with previous reports (Akanbi *et al*., 2017; Nasution *et al*., 2018). Penicillin and ampicillin were shown in this study to have unequal resistance, unlike in the previous research (Akanbi *et al*., 2017). This might have been due to our isolates having evolved more mechanisms of adaptation to ampicillin than to penicillin.

The resistance mechanism for MRSA is believed to be due to the existence of the mecA gene. Our results demonstrated that antimicrobial resistance rates are higher in mecA-positive Staphylococcus isolates compared to mecA-negative ones, aligning with findings from previous studies (Genetie and Abetu, 2020; Gómez *et al*., 2016) and supporting the fact that the MRSA isolates frequently carry resistance genes to other antimicrobial agents (Garoy *et al*., 2019; Girmay *et al*., 2020).

The presence of the mecA gene typically signifies potential resistance to β-lactam antibiotics and serves as a molecular marker for the identification of MRSA (Nasution *et al*., 2018; Eshaghi *et al*., 2017). In this investigation, the PCR product was shown as a 499 bp amplicon in all resistant isolates. A similar result was shown by (Pournajaf *et al*., 2014; Mohammed *et al*., 2018; Girmay *et al*., 2020), wherein the isolates of MRSA have been investigated, truly have a *mec*A gene, which is found in a 20-100 kb region known as the *staphylococcal* cassette chromosome (SCC*mec*) (Dweba *et al*., 2019; Pournajaf *et al*., 2014). MRSA resistance to methicillin and all beta-lactam group antimicrobials is due to changes in regular penicillin binding protein PBP 2 to PBP 2a. Mutation to PBP 2a confirmed that the change in the binding site resulted in lower affinity to beta-lactam group (Havaei *et al*., 202; Abdulghany *et al*., 2014);

therefore, if the bacteria are cultured in medium containing a high concentration of beta-lactams, they continue to exist and grow.

To the best of our knowledge, this is the first research that has simultaneously used a combination of phenotypic and genotypic approaches to confirm the presence and antibiotic resistance profiles of MRSA strains from urban river water samples in the study area.

CONCLUSION

This study is the first to report the occurrence of antibiotic-resistant *S. aureus* on urban river water samples in Gondar Town, Northwest, Ethiopia. This study shows that the PCR assay is an easy and effective method for detecting MRSA from various wastewater samples. On the other hand, concerning the emergence of multidrug-resistant MRSA strains, rapid detection and timely treatment of their infections and contamination help to minimize the mortality and prevent the spread of these organisms. Compared to mecA-negative isolates, the isolates with the mecA gene were shown to be highly resistant to all commonly used antibiotics. In general, the present study indicated that the rivers are potential sources of MRSA infections. Thus, continuous bacteriological assessment of many point sources of waste entering to *Keha* and *Shinta* rivers is ideal for preserving the safety of the rivers. However, multiple studies are required to find additional knowledge about the microbiological quality and treatment efficiency of several hospitals waste water which is mixed with the urban rivers of Ethiopia.

LIST OF ABBREVIATIONS AND ACRONYMS

ATCC43300	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic acid
DW	Dashen factory waste
KHW	*Keha* Hospital waste
KB	*Keha* River before
MRSA	Methicillin-resistant *Staphylococcus aureus*
MGE	Mobile gene element
MSA	Manitol salt agar

MSSA	Methicillin-sensitive *Staphylococcus aureus*
OP	Oxidation pond
PCR	Polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
ATCC	American Type Culture Collection
SB	*Shinta* River before
SCCmec	*Staphylococcal* chromosome cassette mec

CONFLICT OF INTEREST

We declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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