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ORIGINAL RESEARCH

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 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. Isolation of *S. aureus* was conducted following standard morphological and biochemical method and subjected to susceptibility testing to 8 antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected by using the standard PCR method using specific pair of primers. Genomic DNA of the isolates was isolated using DNA Extraction Kit (GenElute^{tm}., USA). Amplification of mecA gene was done by PCR using specific primer for the *mec*A gene. The PCR products were visualized using agarose gel-electrophoresis with 1.5% gel. The results indicated that four (66.7%) Methicillin-Resistant *Staphylococcus aureus* isolates showed to have 499 bp band size of mecA gene. *S. aureus* showed a wide range of resistances, 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%). Four (4/6, 66.7 %) *S. aureus* isolates showed multiple antibiotic-resistant patterns (resistant to four or more antibiotics). The result of this finding concluded that, *S. aureus* isolates with mecA gene were developed more resistant to many antibiotics than with mecA negative isolates. In addition, the present study confirmed that, the treated wastewaters mixed to 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 point sources in the study area. In conclusion, this study's 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

1. INTRODUCTION

The surgeon Sir Alexander Ogston first discovered *Staphylococcus* in 1880 in Aberdeen, Scotland, in pus from a surgical abscess in a knee connection (Puma *et al*., 2017). *Staphylococcus aureus* is gram-positive *cocci* in diameter ranging from 0.5 to 1.5µm, which may or may not contain a capsule of polysaccharides. They are neither motile nor spore forming facultative anaerobes that produce such as catalase and coagulase enzymes (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. Earlier study on *S. aureus* in rivers has associated bathers to 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; Water & Organization, 2006).

Every year, microbial contamination of river waters is predicted to be accountable for millions of gastrointestinal and acute respiratory infections, in addition to several skin infections (Das *et al*., 2017; Hashemi *et al*., 2018). While usually, *S. aureus* is a commensal organism, it was known to be opportunistic. Numerous illnesses, including scalded skin syndrome, abscesses, septicemia, pneumonia, food poisoning, and toxic shock syndrome, may result from invasive infections due to wound invasion (Thapaliya *et al*., 2017).

Misuse of antibiotics for human and animals results an increase in antibiotic resistance and leads to the spread of resistance genes in environmental samples such as hospital wastewater (Liu *et al*., 2016; Tamhankar & Stålsby Lundborg, 2019). Studies have shown that hospital waste water is highly selective and leads to high levels of resistant bacteria released into the natural environment (Galler *et al*., 2018). As confirmed by Brooks., *et al* (Brooks *et al*., 2014) the occurrence of bacteriophages from samples of animal fecal wastes can be environmental vectors for the horizontal transfer of antibiotic resistance genes which results highly resistant pathogenic bacteria.

S. aureus has been rapidly developing resistance to almost any antibiotic drugs (Galler *et al*., 2018). It has been widely reported that *S. aureus* is resistant to antimicrobial drugs, and this resistance played a key role in the treatment's failure (Thapaliya *et al*., 2017). In 1961, the date marking the emergence of Methicillin-resistant *Staphylococcus aureus*, resistance to methicillin suggesting resistance to all beta-lactam agents was first identified. Any *S. aureus* strain known as MRSA is one that 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 is coding penicillin-binding protein 2a (PBP2a) in *S. aureus'* makes the microbe resistance 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 were reported and is possibly due to existence of antimicrobial residues in water and high concentrations of microorganisms that facilitate the exchange of genetic material (Porrero *et al*., 2012; 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 conducted mainly on antimicrobial susceptibility approaches for detection of MRSA, including disk diffusion method. Unfortunately, limited research has been done on bacteriological quality assessments of urban river waters and that of several waste waters. 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 only microbial characterization and identifications of MRSA from clinical samples. Thus, this research work aims to assess the association of mecA gene polymorphism and drug resistance pattern of MRSA isolated from *Keha* and *Shinta* rivers of Gondar town, North West, Ethiopia.

2. MATERIALS AND METHODS

2.1. Study Area Description

The study was carried out in Gondar town from February, 2020 to October, 2020. The study area is placed in Gondar city which is located in the North western Ethiopia, the 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 longitudes with an altitude range of 2000-2200m above sea level and with 20^{o}c annual average temperature, 1172 mm average annual rainfall. According to the 2007 National Population and Housing Census, Gondar consists of a total of 50, 817 housing units (Aneja, 2007).

2.2. Sample Size and Sample Collection

A total of 10 water samples from ten different sampling sites were intentionally chosen from *Keha* and *Shinta* River. In the former river four samples were collected and each sample site was designated as "Khw1 and Khw2: after the junction of hospital waste in to *Keha* river; Kb1 and Kb2: before the junction of hospital waste in to 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 in sterile glass bottles and transported to the laboratory in ice box (Adesoji *et al*., 2019). The bacteriological analysis was carried out in the microbial laboratory and later on, molecular analysis was conducted in the molecular laboratory of the department of biotechnology, Institute of Biotechnology, University of Gondar.

2.3. 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. Using their colonial appearances such as size, shape, color and the differential characteristics such as pigmentation, suspected colonies of *S. aureus* were sub-cultured by quadrant streaking on nutrient agar plates to obtain a pure culture according to guidelines (Faridi *et al*., 2018). Colonies on MSA and blood agar that were yellow and creamy white, respectively, were picked up aseptically and subjected to additional biochemical analysis (Cheesbrough, 2005).

Gram Staining And Biochemical Tests

According to the previous studies (Genetie and Abetu, 2020; Qdais *et al*., 2010), the bacterium (*S. aureus*) was isolated and identified by its colony and cell morphology, Gram staining and biochemical tests (catalase and coagulase tests) (CLSI, 2016).

Antimicrobial Susceptibility Testing

Susceptibility of isolates were done 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 (Huber *et al*.,

2011). Intermediate results were considered as resistant (Seyedmonir *et al*., 2015).

Genomic Dna Extraction

The genomic DNA of S. *aureus* isolates were extracted from pure culture (1.5 ml) grown overnight in Liquid broth using DNA Extraction Kit (GenElute^{tm}., USA) according to manufacturer's instructions. The purity and concentration of isolated Genomic DNA was confirmed by using NanoDrop (Thermo Scientific™, Nano-400. China).

Amplification and Detection of MECA Gene Using PCR Technique

The standard PCR assay was performed using the DNA amplification instrument Master cycler gradient (TECHNE, Germany) to identify MRSA strains. The mecA- specific primer pairs (5'-AAAATCGATGGTAAAGGTTGGC-3', and Reverse, 5'-AGTTCTGGAGTACCGGATTTGC-3') were used for amplification of 499 (bp) fragment (Pournajaf *et al*., 2014). A volume of 2 µL of template DNA was added to a final volume of 20 µL PCR mixture containing 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 were comprised per-denaturation 94 °C for 3 min, denaturation 94°C for 30 sec, annealing 58°C for 30 sec, extension 72°C for 1 min, final extension 72°C for 7 min and final hold 4°C followed by 33 cycles. The amplified products were visualized by gel electrophoresis using 1.5% agarose gels stained with ethidium bromide and its size was determined using 100 bps DNA ladder. Known antibiotic resistances strains of *S. aureus* (ATCC43300) was used as a positive control (Abdulghany and Khairy, 2014).

2.4. Data Analysis

SPSS statistical package version 20 was used to analyze the data obtained from experiments to determine the frequency and percentage of the isolates' resistance and susceptibility to antibiotics.

3. RESULTS

3.1. Isolation and Characterization of Bacterial Isolates

A total of 10 samples were screened; 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-road (100X). The remaining 3 (30%) were gram negative, *cococci* shaped bacteria. The grapes like bacteria 6 (60%) were later identified as *S. aureus* after biochemical test (Table 3). Bacterial isolates that showed morphological characteristics of golden-yellow colonies on nutrient agar, grape-like clusters under a microscope, and the ability to ferment mannitol salt and had beta-hemolytic activity on blood agar (as shown in Tables 1 and 2) were subjected to further molecular characterization and antibiotic resistance tests.

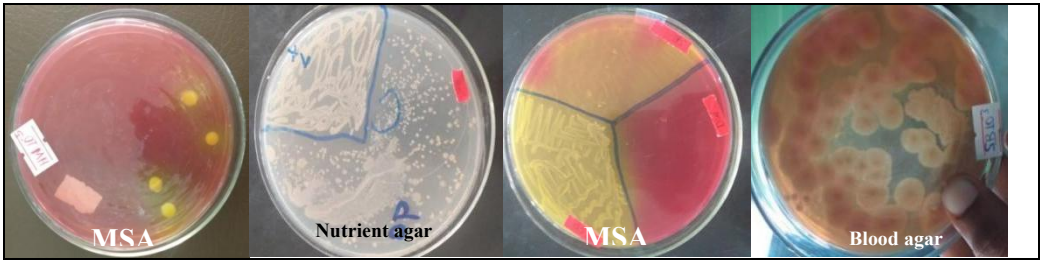

Figure 1: Morphological characterization of isolates on MSA, Nutrient Agar and Blood Agar.

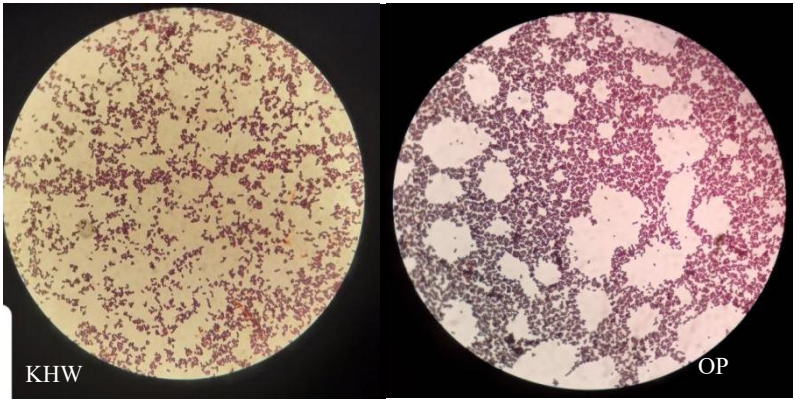

Figure 2: The representative microscopic view of *S. aureus* isolates of *Keha* River after the junctions of University of Gondar referral hospital waste (KHW) and sewage waste samples taken from the oxidation ponds (OP).

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
Dw1	White	Long-Road	Circular	Smooth	Transparent
Dw2	Yellow	Long-Road	Irregular	Smooth	Opaque
Op1	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Op2	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Sb1	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque
Sb2	Golden-yellow	Grape-like clusters	Round	Smooth	Opaque

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

	Media type				
	Mannitol Salt Agar (MSA)		Blood Agar		
Isolates	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, 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.

3.2 Biochemical Test Based Isolation and Identification of Bacterial Isolates

Biochemical tastes were subjected to isolates which were appeared as yellow and golden color colony 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 taste, 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 that a positive result, while (-) sign shows a negative result for isolates to grams reaction, catalase, coagulase, mannitol fermentation and blood hemolysis test.

3.3 Antimicrobial Susceptibility Testing (AST)

Antibiotic susceptibility of six *S. aureus* isolates revealed varying degrees of susceptibility patterns against the antimicrobial agents. Generally, Ciprofloxacin 100% (6/6), Gentamycin 100% (6/6), and Vancomycin 66.7% (4/6), were the most effective antibiotics to *S. aureus* (Table 4). As represented in a bar chart (Figure 4), 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 believed to be mecA gene positive, 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

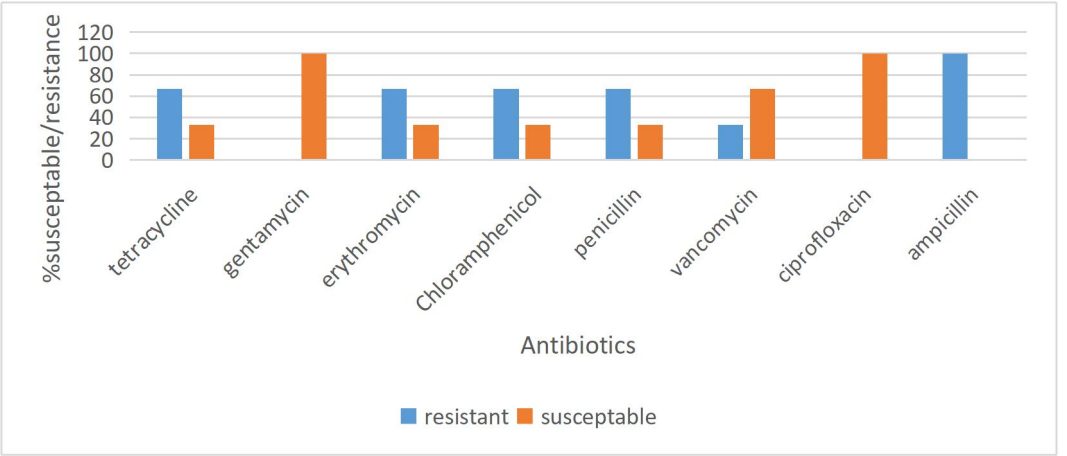

Figure 3: The percentage of antimicrobial resistance profiles of *S. aureus* isolates.

3.4 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 Fig. 4 below.

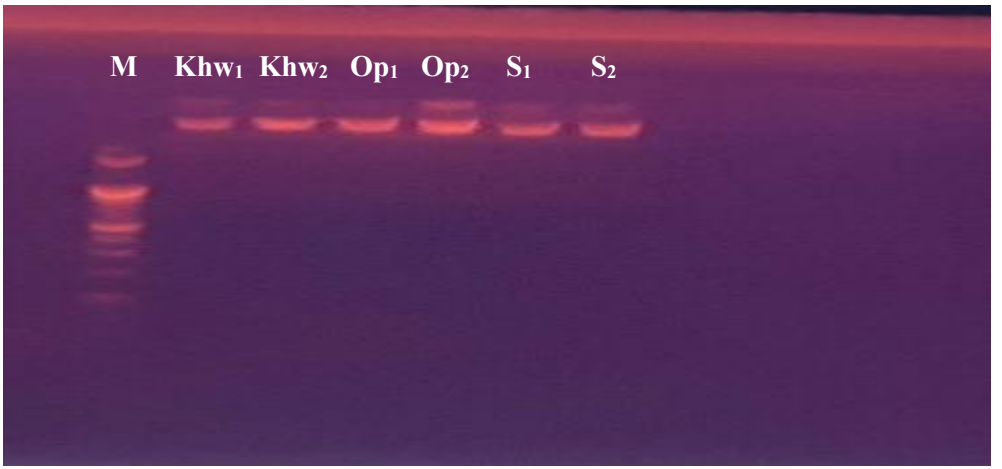

Figure 4: The gel electrophoresis view of genomic DNA extracted from six *S.aureus* isolates and 1000 bp sized molecular marker (HIMEDIA, SwastikDisha Business Park, Mumbai, India) were used.

3.5 PCR-Based Detection of MECA Gene of MRSA

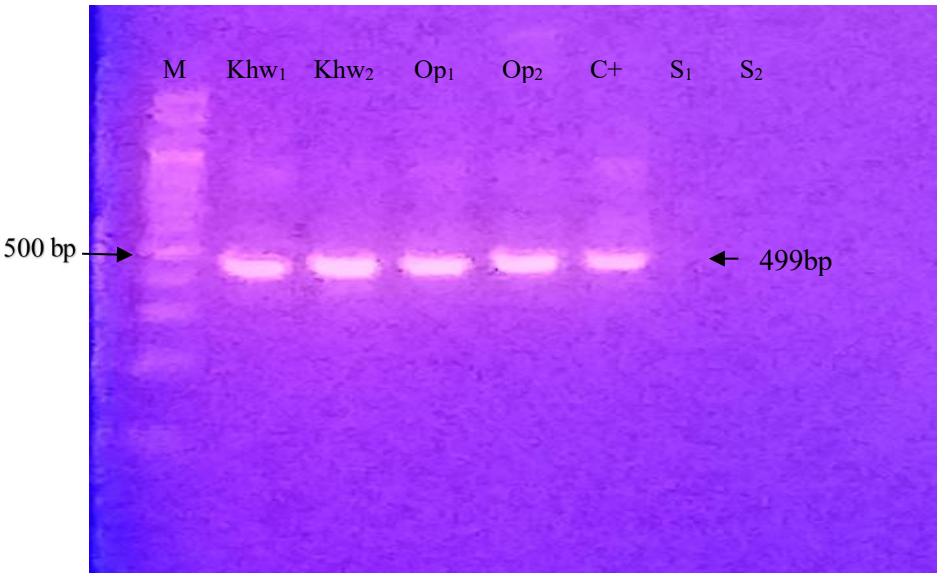

Figure 5: Representative gel showing PCR amplified products of antibiotic resistance mecA gene for selected isolates of *S. aureus.* Lane S1 and S2: mecA negative *S. aureus*; lane Khw1, Khw2, Op1 and Op2: PCR product of mecA gene (499 bp); M: 100 bp size DNA marker (HIMEDIA, SwastikDisha Business Park, Mumbai, India); C+: Known antibiotic resistances strains of *S. aureus* (ATCC43300) (positive control), Using 1.5 % agarose.

4. DISCUSSION

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, are 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.

Results obtained from this study show that the prevalence of MRSA isolates (40%) in urban river water samples 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 in 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 evaluated in this study, four methicillin-resistant *S. aureus* (MRSA)

isolates, 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 bacteria, which were isolated from the explored river water samples (Genetie and Abetu, 2020; Das *et al*., 2017). Six of the *S. aureus* isolates, two and four isolates were isolated from water samples of the *Keha* and *Shinta* rivers, respectively, in the present study, considered primarily as pollutant junction sites of those 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 all commonly used antimicrobials: penicillin (10 µg), ampicillin (10 µg), tetracycline (30 µg), erythromycin (15 µg), and chloramphenicol (30 µg). This finding agreed with previous research that reported the occurrence of multi-drug and methicillin-resistant *Staphylococcus aureus* (MRSA) from all sources of domestic water (Das *et al*., 2017; Genetie and Abetu, 2020; Mohammed *et al*., 2018). Of the isolates evaluated in this study, individual resistances of *S. aureus* to penicillin were extremely high (100%; 6/6), and tetracycline and ampicillin were (66.7%; 4/6), but our finding was unlike the study conducted by 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 the previous study conducted by Gabriella and his colleagues' (Nasution *et al*., 2018), but it was in line with 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, and municipal, and domestic origins (Hatcher *et al*., 2016).

High resistance to these β-lactam antibiotics was expected given that penicillin has been resistant to *S. aureus* 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 resistant to ampicillin may cross-select for resistance to other beta-lactam antibiotics (Garoy et al., 2019; Aziz and Hassan, 2019; Ghaznavi-Rad & Ekrami, 2018). This was observed in our study, as resistance to ampicillin has shown resistance to erythromycin, chloramphenicol, penicillin, and tetracycline, which is similar to that of previously reported (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 findings showed that the rate of antimicrobial resistance between mecA-positive compared to mecA-negative *Staphylococci* is greater. These results are consistent with the other studies (Genetie and Abetu, 2020; Gómez *et al*., 2016) and support the fact that the MRSA isolates frequently carry resistance genes to other antimicrobial agents (Garoy *et al*., 2019; Girmay *et al*., 2020).

The existence of the mecA gene normally to indicate the potential resistance to the beta-lactam group and is used as a marker to identify MRSA. 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), and Girmay *et al*. (2020), wherein the isolates of MRSA have been investigated and truly have a mecA gene, which is found in a 20-100 kb known as staphylococcal cassette chromosome

(SCCmec) (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 ended in lower affinity to the beta-lactam group (Havaei *et al*., 202; Abdulghany *et al*., 2014); therefore, if the bacteria are cultured in a 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.

5. CONCLUSION

This study is the first to report the occurrence of antibiotic-resistant *S. aureus* in urban river water samples in Gondar Town, Northwest Ethiopia. This study shows that 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 the *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' wastewater, which is mixed with the rust 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

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Conflict of interest

The authors do not have a conflict of interest in the publication of this article.

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