CO-OCCURRENCE OF ANTIBIOTICS AND HEAVY METAL RESISTANCE IN MICROBES ISOLATED FROM LAKES, INDIA

Pritha Chakraborty*

* JAIN (Deemed to be University), School of Allied Healthcare and Sciences, Bengaluru, Karnataka, India

corresponding author: Pritha Chakraborty, e-mail: prithachakraborty7@gmail.com

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ABSTRACT

The rising levels of heavy metals and antibiotics in municipal sewage and sludge pose a significant environmental and public health concern. These pollutants exert selection pressure on microbial communities, promoting co-selection of heavy metal and antibiotic resistance in bacteria. In this study, water samples were collected from Lake Agara, HSR Layout, Bangalore, Karnataka, India, to investigate this phenomenon. Mercury concentration was determined using atomic absorption spectrometry (AAS), while the presence of antibiotics was analysed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Mercury-resistant bacteria were isolated using the Mathena criteria after mass seeding and subsequently characterized biochemically and identified molecularly. The mercury concentration was 0.9078 mg/L. LC-MS/MS confirmed the presence of penicillin, streptomycin, and methicillin. Six mercury-resistant bacterial isolates were identified as *Staphylococcus warneri* SAHSPC1, *Staphylococcus pasteuri* SAHSPC2, *Staphylococcus aureus* SAHSPC3, *Pseudomonas citronellolis* SAHSPC4, *Pseudomonas koreensis* SAHSPC5, and *Rheinheimera chironomi* SAHSPC6. Antibiotic sensitivity testing showed that all Staphylococcus and Pseudomonas strains were resistant to at least one antibiotic. These findings highlight the role of polluted water bodies in promoting co-selection and spread of multidrug-resistant bacteria, raising serious implications for environmental and human health.

Keywords: *heavy metal, antibiotic, mercury-resistant bacteria, antibiotic resistance, co-resistance, LC-MS/MS*

INTRODUCTION

In recent years, many research works have been focused on antibiotic resistance (AR) with an environmental basis. There are growing evidence of origin and evolution of antibiotic resistance in different environmental niches [1-4]. There is a need to investigate and comprehend how the environment (soils, waterways, sediments, etc.) can serve as reservoirs of antibiotic-resistant bacteria, as many of the resistance genes discovered in pathogenic bacteria have developed or been acquired from environmental microbial populations [5]. There are reports of bacterial strains resistant to clinically significant antibiotics, including tetracyclines, aminoglycosides, β-lactams, glycopeptides, macrolides, quinolones, streptogramin, and trimethoprim/sulfamethoxazole. According to reports, multidrug-resistant microorganisms are also widespread in the environment [6].

The increase in the occurrence of antibiotic-resistant bacteria is due to the extensive use of antibiotic in medical treatment and agriculture. However, it is also observed that the spread of antibiotic-resistant bacteria can also be due to the influence of heavy metal contamination [7, 8]. In some parts of the world, heavy metals like mercury are a major source of soil contamination. Hg mineral deposits are mainly found as cinnabar (HgS), which is known to survive for up to 1.7 years, degrading the environment [9, 10].

In the presence of Hg, the co-selection of antibiotic resistance and heavy metal resistance appears to be the most obvious of all the heavy metals. Transposons, integron-associated integrases, and plasmids appear to have a close relationship with resistance genes. Bacteria from different environmental niches, such as mine sediments, freshwater microcosms, agricultural soil, wastewater treatment systems [11], and water sediment, have shown a common correlation between antibiotic and mercury resistance. However, a complex network of ecological, evolutionary, and environmental influences shapes the bacterial communities [12, 13].

Mine tailings and drainage, industrial and pharmaceutical wastes, urban wastewater, and agricultural runoff contribute significantly to the widespread presence of heavy metal contamination in environmental reservoirs. Co-resistance and cross-resistance have been identified as the two primary pathways that dominate the link between microbial acquisition of antibiotic resistance and metal resistance [14, 15]. When genes encoding resistance phenotypes are found on the same mobile genetic elements – MGEs – (plasmids, transposons, and integrons), co-resistance takes place. During conjugation, MGEs act as physical carriers of genes between microbes [14].

In India, biological and chemical oxygen demand (BOD and COD) are among the traditional parameters, while for microbial parameters only the total number of coliform bacteria is considered. Antibiotic resistance parameters are not included when determining water quality standard. Because of this, there is a significant lack of available data on occurrence of antibiotic- and heavy metal-resistant bacteria among the important cities in the country. Poor wastewater treatment facilities in cities encourages the direct discharge of untreated waste into water bodies and different environmental niches [16].

In this study, Bangalore was selected because of its major lakes and because is recognized as the fastest growing city in the world, due to a significant increase in population in recent years. Lake Agara in HSR Layout ward, Bommanahalli zone, Bangalore south, was chosen for this study as this lake is situated between Lake Madiwala (upstream) and Lake Bellandur (downstream). The interconnection between the lakes is disturbed by rapid urbanization. The specific objectives of this study include: i.) isolation of mercury-resistant bacteria from lake water, ii.) analysis of resistance/sensitivity of the isolates to clinically important antibiotics, iii.) morphological and molecular characterization of the isolates showing antibiotic resistance, and iv.) analysis of mercury and antibiotics presence in the collected samples.

METHODOLOGY

Sample collection and characterization

Water samples were collected from 4 different locations around the lake in sterile laboratory grade bottles (Tarsons). The collected samples were stored in an ice box and transported to laboratory and stored in a refrigerator at 4°C for further analysis. The samples were filtered through Whatman filter paper to remove sediment particles. The collected water was analysed for temperature and pH.

Isolation of mercury-resistant bacteria

Mercury-resistant bacteria were isolated following a mass seeding process. One ml of water sample was inoculated on nutrient agar plates spiked with 40 µg/ml of $HgCl_2$ according to the Mathena criteria. Isolates grown in the presence of 40µg/ml of $HgCl_2$ are considered as mercury-resistant and were selected for further analysis [16].

Morphological and biochemical characterization of isolates

Morphological characterization of these isolates was done by Gram staining procedure. After performing the staining procedure, the isolates were observed under the microscope at different magnifications. Biochemical characteristics of the isolated mercury-resistant bacterial strains were evaluated. The isolated strains were evaluated for their ability to ferment different sugars such as sucrose, glucose, maltose, lactose and xylose. Indole, Methyl Red, Voges-Proskauer, and Simmons citrate tests (IMViC) were also performed. Other biochemical tests such as motility, Triple sugar iron (TSI), catalase and gelatinase tests were also performed. The bacterial isolates were grown on different media to assess their growth pattern. The genus of the isolated mercury-resistant strains can be determined from these biochemical characterization tests [17].

Molecular identification of isolates

Molecular identification of the mercury-resistant bacterial isolates was carried out using Sanger's method. Bacterial DNA was isolated by suspending colonies from pure culture plate in 100 µl of 1x phosphate-buffered saline (PBS). The DNA was isolated using Chromus Genomic DNA isolation kit. 16S rRNA gene was amplified using forward (5'-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-TCGGTCTGGAAGGTGGTATC-3′) primer. The polymerase chain reaction amplification was carried out in a final volume of 100 µl. Briefly, the amplification reaction containing 100 ng template DNA, 400 ng each of universal primers, 2.5mM Deoxynucleotide Triphosphate (dNTPs), 10× Taq DNA polymerase assay buffer, and Taq DNA polymerase enzyme was performed on a Thermal Cycler ABI2720. The amplification reaction was cycled as follows: initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and final extension at 72 °C for 15 min for 35 cycles. The product was directly sequenced with the primer using ABI 3130 Genetic Analyzer (Chromous Biotech Pvt. Ltd., Bangalore, India). The sequencing result was submitted to the GenBank database of National Center for Biotechnology Information (NCBI) [18].

Assessment of the sensitivity of selected strains to antibiotics

To study the sensitivity of selected bacterial strains to antibiotics, Disc diffusion method was applied. The following antibiotics were used: Ciprofloxacin, Methcilin, Penicillin, Imipenem, Levofloxacin, Steptomycin, Chloramphenicol, Ceftazidime, and Amoxicillin. Muller-Hinton agar plates were prepared and inoculated with 0.1 ml of aliquote from the selected bacterial culture. The antibiotic discs were placed on the agar surface. The plates were incubated at 35-37 °C for 24 h. The plates were observed for the presence or absence of zone of clearance after incubation period [19].

Analytical studies

Detection of mercury in the water samples

The concentration of mercury present in the sample was determined using atomic absorption spectroscopy (AAS). Water sample (100 ml) was transferred into a clean beaker. An equimolar mixture of concentrated HNO_3 and water (1:1) was used to digest the sample, which continued until the volume was reduced to 10 ml using hot plate. After the sample cooled to room temperature, distilled water was added, and volume was adjusted to 100 ml. The sample was then aspirated into an instrument (atomic absorption spectrophotometer) to determine the specific metal (mercury). Results were analysed and presented as mg/L.

Detection of antibiotics in the water samples by LC-MS/MS

Electrospray ionization (ESI) mass spectrometry was performed on a Finnigan TSQ 7000 mass spectrometer operating in single quadrupole mode. The capillary temperature was 200 °C and the ESI voltage was 4.5 keV. The quadrupole was scanned from m/z 100 to 750. The positive-ion mode was used. For LC-MS, the injection volume was 20 µl and samples were introduced via an inlet using a flow rate of 200 µl/min. A continuous gradient system was followed using mobile phase composed of water: acetonitrile: formic acid (5 - 50 %) for 20 min. XEVO-TQD#QCA1232 instrument was used. SUNFIRE C18 column (4.6 mm x 250 mm and 5 µm particle size) was used. Absorbance was monitored at 280 nm. The solvents used were LC-MS grade [20].

RESULTS AND DISCUSSION

Physiochemical analysis of collected water samples

Sixteen collected water samples were analysed for temperature and pH. The water samples were collected four times from January 2023 to August 2023 (Table 1). During the winter season when temperature was relatively lower, the pH was highest. During the summer season, the overall temperature of the Lake Agara water was increased, while the pH decreased. During the monsoon season, the temperature and pH of the water decreased significantly. Fluctuations in pH between the seasons could be due to the rainfall and the entry of untreated sewage water. A study by Shashank et al. [21] has also reported similar findings.

Table 1. Characterization of collected water samples

Month	pH	Temperature
Jan 23	7.3±0.12	25±0.33
Feb 23	7.2±0.39	26±1.01
Mar 23	7.0±0.22	28±0.38
Apr 23	6.8±0.11	28±0.54
May 23	6.9±0.21	27±0.56
June 23	6.8±0.09	25±0.49
July 23	6.5±0.59	24±0.60
Aug 23	6.8±0.12	26±0.23

Isolation of mercury-resistant bacteria

Mercury-resistant bacteria were isolated from two different locations of Lake Agara. The bacterial colonies grown in presence of 40 µg/ml of $HgCl_2$ are considered resistant to mercury according to the Mathema criteria [22]. Increased number of colony forming units (CFU) was observed in 10^{-2} dilution at 40 µg/ml of $HgCl_2$. High number of bacterial colonies in the presence of $HgCl_2$ indicates that exposure to high concentration of mercury present in lake water induces the bacterial resistance to mercury. It also indicates that bacteria may possess intrinsic resistance to mercury. Higher number of colonies was found in the 10^{-2} dilution compared to the 10^{-4} dilution of the collected water samples (Table 2). The number of resistant bacteria in water samples decreased with dilution. This observation suggests that the water of Lake Agara contains bacteria that are resistant to mercury. Six mercury-resistant bacteria isolated from two different locations of Lake Agara were selected for further study. The isolated bacterial colonies were selected based on their ability to grow at a certain concentration of $HgCl_2$ and the differences in the morphology and characteristics of the colonies.

Table 2. Colony forming units of mercury-resistant bacteria isolated from different location of Agara Lake, Bangalore

Location	Colony forming unit at different dilutions (CFU/100 µl)		
	10^{-2}	10^{-3}	10^{-4}
Location 1	47	15	5
Location 2	53	17	8

Morphological and biochemical characterization of isolated bacteria

Morphological characterization of isolated bacteria was carried out by Gram staining. The result showed that three isolates were Gram-positive while the other three bacteria were Gram-negative.

The biochemical tests were performed to identify the genera of the mercury-resistant bacterial isolates. Table 3 shows the glucose fermentation profile of the isolated strains. Isolate 1 and isolate 2 were able to ferment all mentioned sugar sources except xylose, while isolate 3 was able to ferment all five sugar sources. Isolates 4 and 5 were able to ferment only glucose from 5 sugar sources. Isolate 6 was able to ferment only glucose, maltose and sucrose, but not lactose and xylose. Thus, overall, only one isolate was able to ferment all five types of sugar. All isolates were able to ferment at least one of the five sugar sources.

Other biochemical tests included IMViC tests, motility, catalase and gelatinase tests, which are presented in Table 4. Isolates 1 and 3 showed positive results for methyl red and Voges Proskauer test, while they showed negative results for Indole and Simmon citrate test. Isolates 1 and 3 were non-motile and positive for the enzymes catalase and gelatinase. A positive result was observed only for methyl red in case of isolate 2, while it showed a negative result for indole, Voges-Proskauer and Simmon citrate test. Isolate 2 was also non-motile, positive for catalase test and negative for gelatinase enzyme. Isolates 4 and 5 showed a similar result, where only Simmon citrate test was positive and the other tests in IMViC profile were negative. Both isolates were motile and a positive result was observed for gelatinase and catalase enzyme. Isolate 6 was negative for all IMViC profile tests and showed a positive result for catalase and gelatinase and was found to be motile. The observation showed that the isolates belonged to the non-coliform group of bacteria.

Growth patterns of all the mercury-resistant isolates on differential and selective media were also evaluated (Table 5). Similar result was observed in case of isolates 1, 2 and 3. All showed the presence of colonies on TSI, MacConkey agar and Mannitol salt agar, but there was no growth on the EMB (Eosin Methylene Blue agar) and cetrimide agar. Isolates 4 and 5 had no colonies on TSI and showed colourless colonies on MacConkey agar and EMB agar. No growth was observed on Mannitol salt agar, and colonies appeared on Cetrimide agar. Isolate 6 grew only as colourless colonies on MacConkey agar and EMB agar. It showed no growth on TSI, Cetrimide and Mannitol salt agar.

Pink colonies on the MacConkey agar, without the crystal violet, mostly indicates that the bacteria belong to the *Staphylococcus* genera. It also suggests that it can ferment lactose present in the media, which changes the pH of the agar to an acidic level causing the colonies to turn pink. This observation is supported by the data presented in Table 3, which shows that isolates 1, 2, and 3 were able to ferment lactose. The positive result on the Mannitol salt agar showed that the isolates can grow at a higher salt concentration. The colourless colonies on the MacConkey agar and the EMB agar showed that isolates 4, 5, and 6 isolates were not able to ferment lactose (supported by data presented in Table 3) and mostly belong to the gram-negative group of bacteria. No growth of isolates 4, 5, and 6 was observed on the TSI agar, which indicates that these isolates do not belong to the *Enterobacteriaceae* family. Isolates 4, 5 and 6 did not show growth on the Mannitol salt agar, which indicates that these isolates cannot survive higher salt concentration. Only isolates 4 and 5 showed growth on the cetrimide agar, which indicates that these isolates could belong to the genus *Pseudomonas*.

Table 3. Details of sugar fermentation of mercury-resistant isolates

Serial no	Isolates	Glucose	Lactose	Maltose	Sucrose	Xylose
1	Isolate 1	+	+	+	+	-
2	Isolate 2	+	+	+	+	-
3	Isolate 3	+	+	+	+	+
4	Isolate 4	+	-	-	-	-
5	Isolate 5	+	-	-	-	-
6	Isolate 6	+	-	+	+	-

Table 4. Biochemical characterization of isolated mercury-resistant isolates

Isolates	Indole	Methyl red	Voges-Proskauer	Citrate	Gelatinase	Catalase	Motility
Isolate 1	-	+	+	-	+	+	-
Isolate 2	-	+	-	-	-	+	-
Isolate 3	-	+	+	-	+	+	-
Isolate 4	-	-	-	+	+	+	+
Isolate 5	-	-	-	+	+	+	+
Isolate 6	-	-	-	-	+	+	+

Table 5. Growth pattern of isolated mercury-resistant bacteria on different media

Isolates	TSI	MacConkey Agar	Eosin methylene blue Agar	Cetrimide Agar	Mannitol salt agar
Isolate 1	A/A	Pink colonies (Without crystal violet)	-	-	+
Isolate 2	A/A	Pink colonies (Without crystal violet)	-	-	+
Isolate 3	A/A	Pink colonies (Without crystal violet)	-	-	+
Isolate 4	-	Colourless colony	Colourless colony	+	-
Isolate 5	-	Colourless colony	Colourless colony	+	-
Isolate 6	-	Colourless colony	Colourless colony	-	-

Molecular identification

Molecular identification of bacterial isolates was performed using the 16S rRNA sequencing method. After PCR amplification, 16S rRNA sample of bacterial isolates were observed through agarose gel electrophoresis, which showed a single band of 500bp. The sequence was further analysed by BLAST to identify the bacterial strain. The 16S rRNA sequence was then submitted to Genbank of National Centre for Biotechnology Information (NCBI). Details of the identified bacterial strain and accession number received from NCBI are presented in Table 6. The 16S rRNA sequencing showed that the mercury-resistant bacteria belong to genera *Staphylococcus, Pseudomonas and Rheinheimera*. Mercury resistance in the genera *Staphylococcus, Pseudomonas and Rheinheimera* is supported by findings of Acharyya et al. [23], Lo et al. [24] and Zhao et al. [25]. The finding suggests that these isolates can be used for bio-removal of mercury from contaminated water. The phylogenetic tree was constructed using CLUSTALW software using neighbour-joining method. Individual phylogenetic trees of the isolated strains are shown in Figure 1. Phylogenetic tree in Figure 2 highlights the evolutionary distance or relationship between the mercury-resistant isolates. It reflects the nucleotide sequence-based relatedness between the isolates. The external nodes indicate the point of divergence while the clusters present the evolutionary relationship.

Table 6. Molecular identification of mercury-resistant bacterial isolates

Serial no	Isolate	Accession no	Strain name
1	Isolate 1	PQ436492	*Staphylococcus warneri* strain SAHSPC1
2	Isolate 2	PQ436493	*Staphylococcus pasteuri* strain SAHSPC2
3	Isolate 3	PQ653735	*Staphylococcus aureus* strain SAHSPC3
4	Isolate 4	PQ653575	*Pseudomonas citronellolis strain SAHSPC4*
5	Isolate 5	PQ653571	*Pseudomonas koreensis* strain SAHSPC5
6	Isolate 6	PQ436494	*Rheinheimera chironomi* strain SAHSPC6

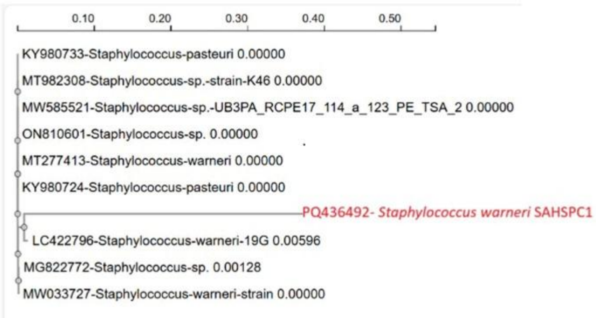

Figure 1a. Phylogenetic tree of SAHSPC1 isolate

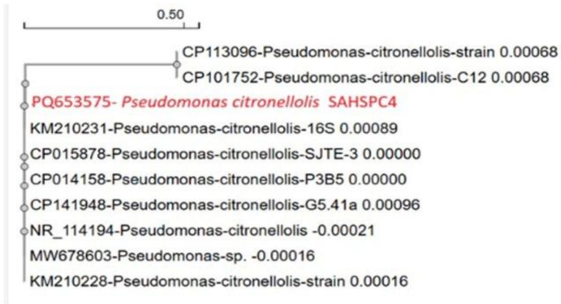

Figure 1d. Phylogenetic tree of SAHSPC4 isolate

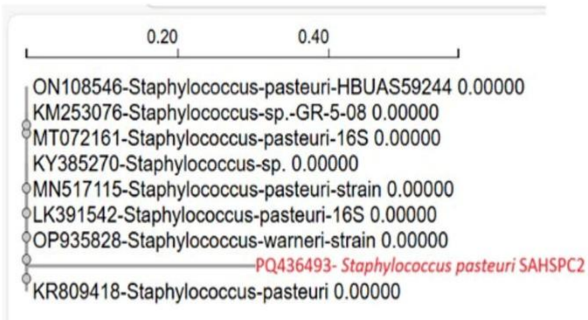

Figure 1b. Phylogenetic tree of SAHSPC2 isolate

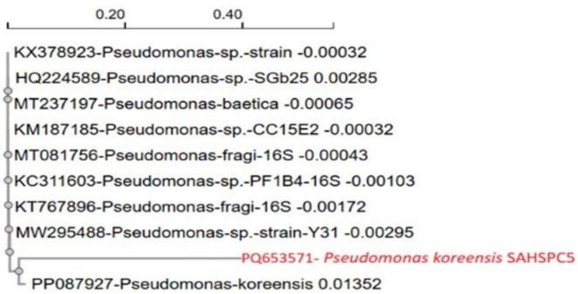

Figure 1e. Phylogenetic tree of SAHSPC5 isolate

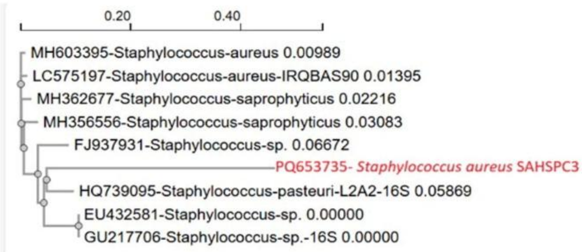

Figure 1c. Phylogenetic tree of SAHSPC3 isolate

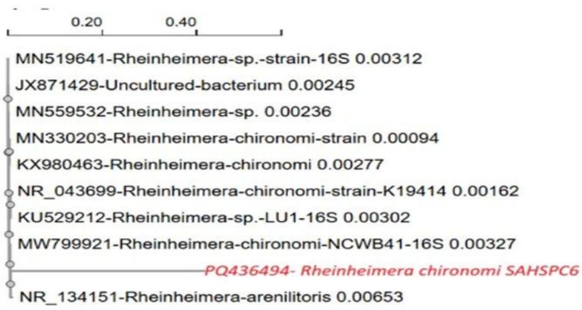

Figure 1f. Phylogenetic tree of SAHSPC6 isolate

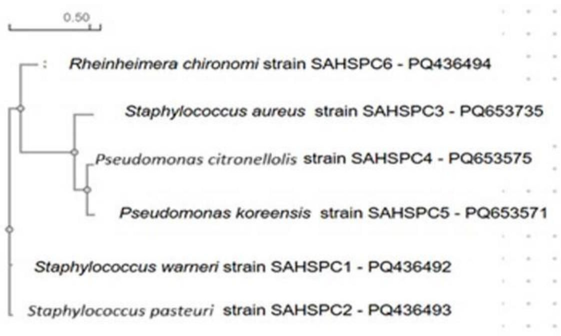

Figure 2. Combined phylogenetic tree of all six mercury-resistant isolates

Antibiotic sensitivity profile of mercury-resistant bacteria

The sensitivity of all six mercury-resistant bacteria to nine different antibiotics was evaluated. The antibiotics were selected based on the frequency of their use. Table 7 and Figure 3 show the antibiotic sensitivity profile of all six isolates. Five bacterial isolates did not show a clear zone against methicillin, which indicates that the growth of SAHSPC1, 2, 3, 4, and 5 was not inhibited by the antibiotic methicillin. Apart from methicillin, SAHSPC 4 did not show a clear zone against penicillin. SAHSPC 5 did not show a clear zone against penicillin and streptomycin, nor against methicillin. SAHSPC 6 showed sensitivity to all antibiotics used due to the presence of a clear zone against all antibiotics used. The absence of a clear zone against any antibiotic for selected isolate indicates that the isolate may possess resistance to the antibiotics. From the above data, it can be concluded that five mercury-resistant isolates are resistant to at least one antibiotic. One the other hand, none of the isolates showed resistance to Ciprofloxacin, Ceftazidime, Imipenem, Amoxicillin, Levofloxacin, and Chloramphenicol.

Methicillin resistance in the genus *Staphylococcus* is mediated by altered expression of the penicillin-binding protein, which is coded by *mec*A and *mec*C present in SCC*mec* (Staphylococcus Cassette Chromosome *mec*). Among 14 of such SCC*mec* cassettes, five such mobile elements (SCC*mec* I, II, III, IV, V) are most commonly found in methicillin-resistant Staphylococcus. Studies have shown that methicillin-resistant Staphylococcus, which also carries SCC*mec* III, exhibit resistance to mercury [26]. Another mobile genetic element known as the arginine catabolic element (ACME) was identified in methicillin-resistant *Staphylococcus sp*, which is 31bp long and shows similarities to SCC*mec*. ACME is also known to form composite island between SCC*mec* and SCC associated genes. The copper and mercury resistance genes known as COMER are located in ACME adjacent to SCC*mec* III genetic element. This mobile element is believed to be responsible for co-resistance to methicillin and heavy metals (mercury) in Staphylococcus sp. Staphylococcus carrying the *mec* gene and mobile genetic elements such as ACME and COMER are able to survive under unfavourable conditions [27].

Table 7. Antibiotic sensitivity profile of isolated mercury-resistant bacteria

Isolate/Antibiotic	CIP	CAZ	MET	PEN	IPM	AMX	LFX	STR	CHL
SAHSPC1	S	S	R	S	S	S	S	S	S
SAHSPC2	S	S	R	S	S	S	S	S	S
SAHSPC3	S	S	R	S	S	S	S	S	S
SAHSPC4	S	S	R	R	S	S	S	S	S
SAHSPC5	S	S	R	R	S	S	S	R	S
SAHSPC6	S	S	S	S	S	S	S	S	S

* CIP - Ciprofloxacin, CAZ - Ceftazidime, MET - Methcilin, PEN - Penicillin, IPM - Imipenem, AMX - Amoxicillin, LFX - Levofloxacin, STR - Steptomycin, CHL – Chloramphenicol, R- resistant, S- sensitive

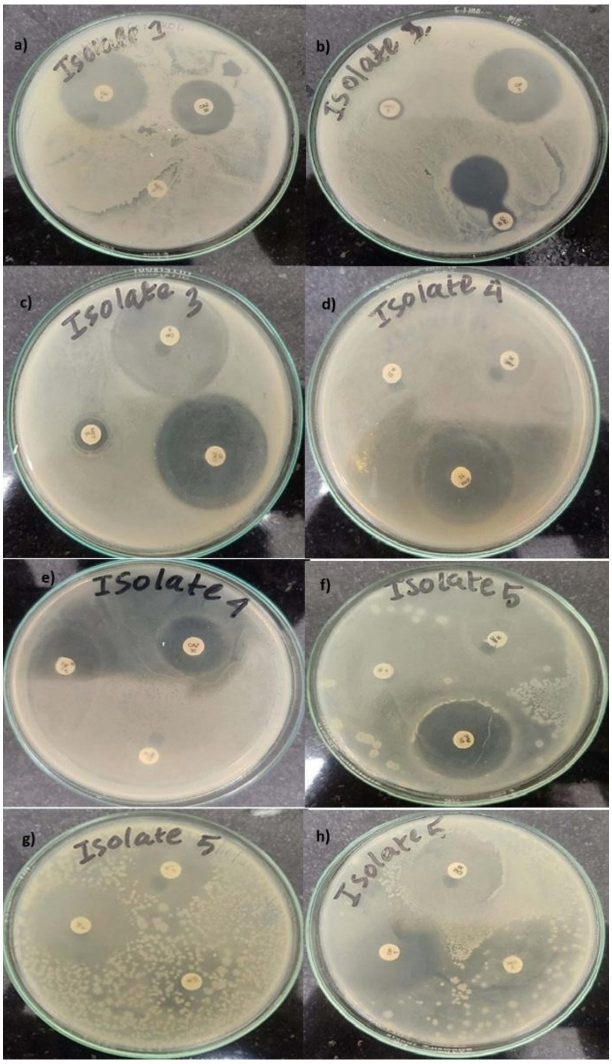

Figure 3. Antibiotic sensitivity of five mercury-resistant bacteria: a) Isolate 1 did not show a clear zone against Methicillin (MET), b) Isolate 2 did not show a clear zone against Methicillin (MET), c) Isolate 3 did not show a clear zone against Methicillin (MET), d) Isolate 4 did not show a clear zone against Penicillin (PEN), e) Isolate 4 did not show a clear zone against Methicillin (MET), f) Isolate 5 did not show a clear zone against Penicillin (PEN), g) Isolate 5 did not show a clear zone against Streptomycin (STR), h) Isolate 5 did not show a clear zone against Methicillin (MET)

A study by Devarajan et al. [28] reported that 35-60 % of *Pseudomonas sp*. isolated from aquatic environment of India were resistant to multiple antibiotics. Among them, 29 % of isolates were categorized under extended spectrum beta lactamase (ESBL) and New Delhi Beta lactamase (NDM). The presence of NDM and ESBL genes in the isolate suggests that treated or untreated sewage from hospitals and urban environments is a source of antibiotic-resistant bacteria in water bodies, which is further influenced by temperature and climate conditions. Hancock et al. [29] have reported that some *Pseudomonas sp*. exhibit resistance to aminoglycoside group of antibiotics, including streptomycin. The main mechanism can be either intrinsic, acquired, or adaptive. Intrinsic resistance is achieved by expelling the antibiotics out of the cell by efflux pump or by enzymes that inactivates other enzymes. Acquired resistance is achieved by horizontal gene transfer or mutation. Adaptive resistance is manifested by biofilm production that acts as a barrier that prevents the entry of antibiotics.

Analytical studies

Mercury level in water sample

The mercury level in the collected water sample was evaluated to determine whether the heavy metal is present in the lake water. The atomic absorption spectrometry result showed that the level of mercury present in lake water was 0.9078 mg/L. In 2014, the Lake Development Authority (LDA) conducted a survey to assess the mercury level in 15 major lakes of Bangalore city, among which was Lake Agara. The findings showed that the average level of mercury present in the lake was approximately 1.187 mg/L. According to the Pollution Control Board standard, a mercury concentration of up to 0.01 mg/L is tolerated. Other studies have also reported presence of different heavy metals in Lake Agara in high concentrations. A study by Shashank and Krishnakumar [21] have also reported the presence of different heavy metals like cadmium, copper, zinc, chromium and lead at an average concentration of 0.1mg/L, 0.30 mg/L, 0.15 mg/L, 0.08 mg/L and 0.67 mg/L. Although the study does not consider the concentration of mercury in the water of Lake Agara, it was determined that the heavy metal pollution of Lake Agara could be due to the discharge of treated sewage water from sewage treatment plant. The concentration of different heavy metals is increasing significantly every year. The mercury concentration found in Lake Bellandur in January 2018 was 0.19 mg/ml, which was increased to 0.61mg/ml by May 2019. Mercury is highly capable of causing harm and toxicity when combined with other elemental forms. Mercury can bind strongly with sulphur and thiol groups in enzymes present in human body, inhibiting metabolic reactions which causes enzymes inactivation and harmful effect on human health [30].

Presence of antibiotic in collected water sample

The water sample was analysed for the presence of antibiotic to which the bacteria has shown resistance. Three antibiotics, penicillin, streptomycin and methicillin, were identified by LC-MS/MS. The peaks identified at 326.6531, 242.5657, 303.4861 indicate the presence of antibiotics penicillin, methicillin and streptomycin in the chromatogram shown in Figure 4. A study by Skariyachan et al. [30] showed that antibiotics penicillin, methicillin and streptomycin were present in the water of Lake Bellandur at the concentration of 5.5 and 10 mcg/ml, along with other widely used antibiotics. Another study by Lata and Mohan [31] reported faecal *Escherichia coli* strains that were 100 % resistant to tetracycline and penicillin. Antibiotics can accumulate and remain in the environment for years. The relatively high concentration of antibiotics in lake water and emergence of antibiotic-resistant bacteria is directly related to overuse or misuse of these antibiotics. The lack of regulatory measures makes antibiotics easily available to the public. Antibiotic misuse, along with poor management of municipal and hospital waste, increases the resistance of several bacteria to multiple antibiotics, promoting multidrug resistance in bacteria from different environmental niches.

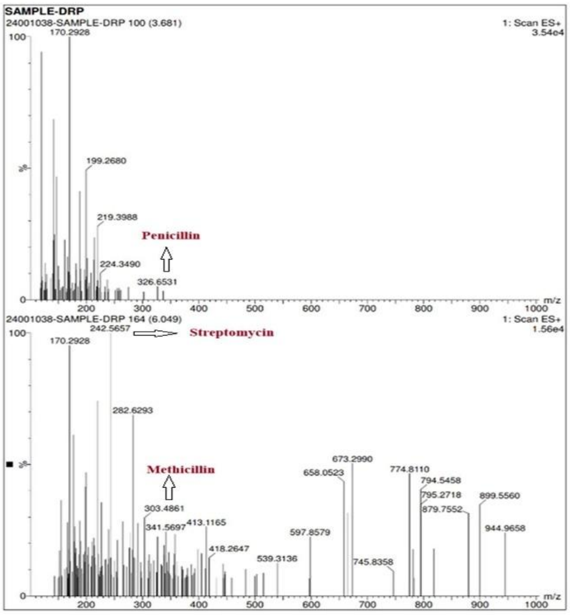

Figure 4. Identification of antibiotics by LC-MS/MS

CONCLUSION

In this study, water sample was collected from Lake Agara, Banglore, Karnataka, India. The collected water was analysed for mercury-resistant bacteria. Six mercury-resistant bacteria were identified, and their antibiotic sensitivity was evaluated. Five mercury-resistant isolates (*Staphylococcus warneri, S. pasteuri, S. aureus, Pseuodomonas citronellosis, P. koreensis*) showed resistance to at least one antibiotic. All five isolates showed resistance to methicillin, a widely used beta-lactam antibiotic. *Pseudomonas citronellosis* showed resistance to penicillin in addition to methicillin, while *P. koreensis* showed resistance to streptomycin in addition to methicillin. Antibiotic sensitivity analysis showed the presence of methicillin-resistant staphylococcus and multidrug-resistant Pseudomonas sp. Both pathogens are classified as priority-1 category pathogen by World Health Organization (WHO). The mercury concentration in the collected water sample was found to be higher than the value prescribed by standard. Methicillin, penicillin and streptomycin were also identified in the water sample, indicating their high concentration. Since this study is based on the laboratory findings, extensive sampling and further characterization are required to comprehend the extent of heavy metal and antibiotic pollution in the Bangalore lakes. This study provides a foundation for future research that can contribute to reducing pollution of freshwater ecosystem.

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