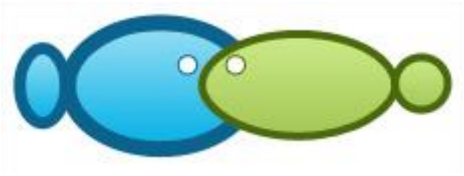

Isolation and characterization of mercury-resistant bacteria from coastal waters of North Sulawesi and their antibiotic resistance profile

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Abstract. Excessive levels of highly hazardous heavy metals are present in the environment. One of the heavy metals that pollutes seawater is mercury (Hg), which is brought on by anthropogenic activities that release debris into the coastal waters. Both high and low concentrations of mercury are detrimental to human health. Microorganisms known as mercury-resistant bacteria can live and thrive in situations where high concentrations of the poisonous heavy metal is present. Mercury-resistant bacteria can help or hurt aquatic conservation. These bacteria can reduce mercury pollution by degrading and detoxifying mercury compounds. Mercury exposure can harm aquatic organisms, ecosystems, and humans. This study was conducted in the coastal regions of Manado Bay, Totok Bay, and Likupang waters in North Sulawesi whereas, the Manado coastal area has an urban background and dense population, the Ratatotok coastal area has a background of community mining that uses mercury, and the Likupang waters area is located away from the residential area. This study aims to isolate and identify mercury-resistant bacteria from the coastal waters of North Sulawesi. From the results of the study, it can be concluded that the bacteria resistant to mercury and isolated from the coastal waters of North Sulawesi are *Pseudomonas hibiscicola*, *Elizabethkingia anophelis*, and *Acinetobacter pittii*. Effective management and reduction of mercury-resistant bacteria can help reduce the negative impact of mercury pollution on aquatic ecology and resource conservation. This research contributes by identifying specific microbes in coastal waters and their resistance to heavy metals, revealing resistance patterns and their implications for public health, and informing for the development of mitigation strategies.
Key Words: heavy metals, human health, water quality.

Introduction. Bacterial resistance to heavy metals has been observed in coastal waters, along with the concurrent emergence of antibiotic resistance in bacterial populations. Resistance to heavy metals and antibiotic resistance can be selected simultaneously in an ecosystem contaminated with heavy metals (Chen et al 2019a; Qiao et al 2018).

Heavy metals are commonly released into coastal waters through various anthropogenic activities, including industrial waste discharge, agricultural runoff, and urbanization, leading to increased concentrations of these metals in the environment (Kepel et al 2018). Studies examining the heavy metal contamination of coastal waters were conducted in Bohai Bay and Yellow Sea, China, and Nizampatnam Bay and Lankevanidibba, East Coast of India (Han et al 2021; Lakshmanna et al 2022; Tian et al 2020). Compared to Manado Bay and Totok Bay, the Likupang waters sampling site, located away from residential areas, had comparatively low heavy metals concentrations (Mantiri et al 2019). Those heavy metals can accumulate in the water, sediment, and aquatic organisms (Juniardi et al 2022).

Bacteria in coastal waters with high concentrations of heavy metals have been shown to acquire mechanisms to withstand the harmful effects of these metals, and this resistance can also confer cross-resistance to antibiotics. When comparing various locations, it is observed that soils and sediments have higher levels of heavy metals and

antibiotic resistant bacteria in comparison to the water layer (Nguyen et al 2019). According to a theory, heavy metals encourage the environment's antibiotic resistance selection and vice versa via cross-, or co-regulation of resistance pathways (Jiang et al 2020; Li et al 2017). Both antibiotic resistance genes (ARGs) and heavy metal resistance genes (MRGs) in bacteria can be activated in response to heavy metal stimulation (Chen et al 2019a).

When bacteria in coastal waters are resistant to both heavy metals and antibiotics, it makes people worry that these resistant bacteria could cause infections that are hard to treat with regular medicines. Misuse and overuse of antibiotics have increased the prevalence of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG), reducing the effectiveness of antibiotics as treatments for bacterial infections in humans and animals (Qiao et al 2018; Sanganyado & Gwenzi 2019).

The combination of heavy metal pollution and antibiotic use in coastal environments creates selective pressures that favour the survival and proliferation of multidrug-resistance bacteria, causing a big threat to the health of people and the environment. Limited information is available on using antibiotic resistance genes (ARGs) as novel indicators for wastewater contamination in coastal environments (Zhang et al 2020). For risk assessment techniques based on enhanced antibiotic detection technologies and antibiotic-resistant bacteria in water, monitoring antibiotic resistance in aquatic environments is essential (Liguori et al 2022).

Material and Method. This study was conducted on the waters of the coastal area of North Sulawesi from August 2022 to February 2023. The sampling takes part in the coastal areas of Manado Bay (1°27’34.5’’N, 124°48’38.0’’E), Totok Bay (0°85’66.72’’N, 124°70’50.50’’E) and Likupang waters, North Minahasa (1°67’59.58’’N, 125°07’37.0”E) (Figure 1).

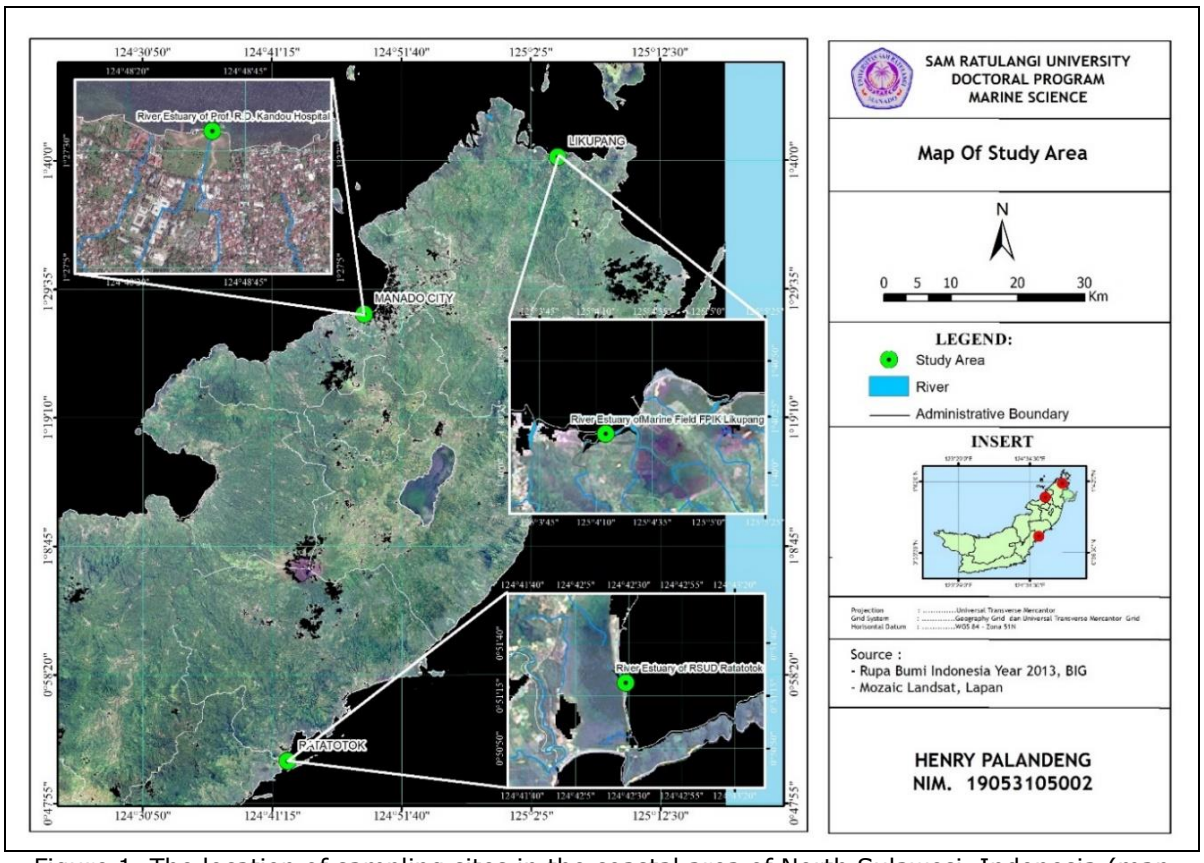

Figure 1. The location of sampling sites in the coastal area of North Sulawesi, Indonesia (map generated using Arcmap 10.8).

Water samples were collected using a 500ml vertical water sampler from a depth of approximately 2 meters below sea level per standard method specifications and kept in sealed bottles at 4°C. Then the samples were analysed at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University.

Based on observations of cell morphology, gram staining, and biochemical tests, including the starch fermentation with gas, glucose, and maltose/lactose, the motility assessment, the Indole test, lysine, citrate development, and catalase, bacteria were identified for physical and biochemical characterization. While identifying bacteria, one form of bacterium is distinguished from another using pre-existing categorization criteria. Bergey's Manual of Determinative Bacteriology lists traits of recognized microorganisms as a reference for recognizing microorganisms (Holt & Williams 2010).

Bacterial culture selection was carried out and tested for mercuric chloride ($HgCl_2$) resistance; this selection was carried out by inoculating the sample bacterial culture in solid selection media containing $HgCl_2$ with concentrations of 250, 500, 750, and 1000 ppm then raised at 37°C for 24 hours, and kept at 40°C. Counts of expanding colonies were made (Dwyana & Fahruddin 2012).

Molecular characterization using the 16S rRNA gene of bacteria was amplified by polymerase chain reaction using universal bacterial primers 27F and 1492R under the following thermocycling environments: 3 min at 95°C for preliminary denaturation, followed by 30 cycles of 2 sec at 98°C for denaturation, 15 sec at 62°C for annealing, and 1 min at 72°C for extension; the last cycle was flown for 1 min at 72°C.

The web program Basic Local Alignment Search Tool (BLAST) (NCBI 2023) was used to compare the results to GenBank and ribosomal RNA. For phylogenetic analysis, the 16S rDNA sequences were compared to related sequences using the GenBank database (NCBI 2023).

Tables and graphs were used to describe the data analysis. The data are described under each parameter's and procedure's observations.

Results and Discussion. Table 1 summarizes the physicochemical appearances of the three analyzed locations, including temperature, pH, conductivity, total dissolved solids (TDS), and salinity.

Table 1

Physicochemical coastal water characteristics

Parameter	*Manado Bay*	*Totok Bay*	*Likupang*
Temperature (°C)	25.6	29.0	29.4
pH	7.45	7.39	7.65
Conductivity (µS/cm)	52.2	54.5	52.7
TDS (ppm)	26.6	27.3	26.4
Salinity (ppt)	33.4	33.3	32.4

In Manado Bay, the result for physico-chemical coastal water characteristics show temperature was 25.6°C, the pH was 7.45, the conductivity was 52.2 µS/cm, TDS was 26.6 ppm, and the salinity was 33.4 ppt. The physico-chemical coastal water characteristics at Totok Bay such as temperature was 29.0°C, the pH was 7.39, the conductivity was 54.5 µS/cm, TDS was 27.3 ppm, and the salinity was 33.3 ppt. In Likupang waters, the temperature was 29.4°C, pH was 7.65, conductivity was 52.7 µS/cm, TDS aas 26.4 ppm, and salinity was 32.4 ppt.

The findings give information on the water quality at three distinct places in North Sulawesi, Indonesia: Manado Bay, Totok Bay, and Likupang. Each location's temperature, pH, conductivity, TDS, and water salinity are listed. The pH levels in all three places are within the usual range for seawater, which is between 7 and 8. Temperatures in Totok Bay and Likupang are greater than in Manado. The conductivity and TDS measurements at all three locations are comparable, indicating that the water contains comparable quantities of dissolved salts. The salinity values of all three places are similar, with Likupang having the lowest salinity. The historically gold-producing Ratatotok Subdistrict

in North Sulawesi has impacted the environment by the placement of undersea tailings, leading to concerns about elevated levels of arsenic and mercury in fish consumed by local residents. Studies have been conducted to evaluate the dietary exposure to arsenic and mercury in the region and to measure the concentrations of these elements in marine fish caught by local fishermen and sold at local fish markets (Bentley & Soebandrio 2017a,b).

Table 2

Coastal water results of the gram stain, motility test, and biochemical test used to identify bacteria

		Isolate code		
		M1.2	*T1.5*	*T1.4*
Morphological Test				
Gram stain		–	–	+
Shape		bacillus	bacillus	coccus
Physiological trial and biochemistry				
Motility		–	–	–
Indole		+	–	+
H_2S		–	–	–
Carbohydrate fermentation	Gas	–	+	+
	Glucose	+	+	+
	Maltose/lactose	+	+	+
Lysine		+	–	+
Citrate		+	+	+
Catalase		+	+	+
Identification results		*Escherichia coli*	*Bacillus* sp.	*Staphylococcus* sp.

Bacteria were identified (Table 2) using morphological analysis (gram staining) and biochemical activity testing (TSI, indole, catalase, and carbohydrate fermentation). Based on their distinct biochemical traits, the bacteria were recognized as *Escherichia coli* for isolate M1.2, *Bacillus* sp. for isolate T1.5, and *Staphylococcus* sp. for isolate T1.4. Incubation and storage followed the bacteria's growth in nutrient broth media and nutrient agar with mercury addition. The variations in biochemical functions suggest that the bacteria belong to various species or strains. Test results of growth for mercury-resistant bacteria isolated from coastal waters in Manado Bay (M1.2), Totok Bay (T1.5), and Likupang (T1.4) are revealed in Table 3.

Table 3

Test outcomes from growth of mercury-resistant bacteria

No.	*$HgCl_2$ (ppm)*	*Test results*			*Explanation*
		M1.2	*T1.5*	*T1.4*	
1	250	+++++++	+++++++	+++++++	Growth present from this turbidity
2	500	++++	++++	++++	Much growth, but still cloudy from 250 ppm
3	750	+++	+++	+++	Still growing
4	1000	+	+	+	Still growing

The results of bacterial isolation with nutrient broth showed that bacteria could grow in the three isolates from each sample point (see also Chasanah et al 2018). The resistance test showed that all bacterial growth in each isolate M1.2, T1.5, and T1.4 from each sample point from Manado Bay, Totok Bay, and Likupang coastal waters, respectively, was very much visible from its turbidity at a specific density of 250 ppm $HgCl_2$. Although the growth was slower than that of bacteria on media with a $HgCl_2$ level of 250 ppm, all bacteria could survive well even at a concentration of 500 ppm HgCl2. When the

concentration of $HgCl_2$ was increased to 750 and 1000 ppm, the isolates could still grow, even though the growth was negligible.

The nutrient broth-based bacterial isolation results revealed that bacteria could thrive in all three isolates from each sample location. At a concentration of 250 ppm $HgCl_2$, all bacterial proliferation was visible in each isolate from each sample point. All bacteria could persist even at a concentration of 500 ppm $HgCl_2$, although their growth was slower than on media containing 250 ppm $HgCl_2$. Even though the rate of growth slowed when the $HgCl_2$ concentration was raised to 750 and 1000 ppm, the isolates continued to grow. Still, the results show that the bacterial isolates from the seawater samples are immune to $HgCl_2$ at high concentrations (Sanz-Sáez et al 2020).

These results could change how $HgCl_2$ is used as a disinfectant or in other business settings (Mulamattathil et al 2014). It is essential to keep in mind that geographic location and other factors, such as industrial activities, agricultural runoff, and natural sources, might affect patterns of antibiotic resistance and the predominance of these microorganisms (Deb et al 2020; György et al 2021; Nusrat et al 2023; Onajobi et al 2020).

At different ppm levels of mercury ($HgCl_2$) on nutritional agar media, a bacterium is said to be mercury-resistant if it can thrive in mercury. Many of these bacteria can live in an environment covering mercury ($HgCl_2$) with levels of 20 ppm or more and are classified as high mercury-resistant bacteria (Fatimawali 2015).

All three isolates, M1.2, T1.5, and T1.4, from each sample point from Manado Bay, Totok Bay, and Likupang waters, respectively, could support bacterial growth, according to the results of the bacterial isolation using nutrient broth. All bacteria, *Escherichia coli* for M1.2, *Bacillus* sp. for T1.5, and *Staphylococcus* sp. for T1.4, were identified as "very mercury-resistant bacteria" after the resistance test revealed that they could all continue to grow and live even in environments with high concentrations of $HgCl_2$ (up to 1000 ppm). The bacteria could survive in a setting where the mercury concentration was 250 ppm or higher.

Understanding mercury-resistant bacteria can help manage and reduce mercury contamination in coastal ecosystems. It has been discovered that mercury-resistant microorganisms are effective in bioremediation treatment processes for mercury-contaminated soil, water, and air (Wang et al 2020). The ability of these microbes to tolerate salinity is crucial for coastal ecosystems (Pushkar et al 2019). Mercury resistance is one of the eubacteria's most frequently detected phenotypes (Barkay et al 2003). Understanding mercury-resistant bacteria and their function in reducing mercury contamination can therefore be an essential topic of discussion for scientists, policymakers, and environmentalists concerned with preventing mercury pollution in coastal ecosystems.

Effective mercury management and reduction can affect aquatic ecology and resource conservation (Hsu-Kim et al 2018). Environmentally-native microorganisms have been shown to have a beneficial effect on mercury pollution remediation through reduction and other methods (Zhao et al 2023). Bacteria have devised numerous mechanisms to resist mercury, including transforming mercury's highly reactive cationic form into volatile, less toxic forms (Barkay et al 2003). Compared to bacteria that live on land, sea bacteria are better at getting rid of metals because they have metal-resistant alleles (Joshi et al 2022). Detoxification of mercury is one method for reducing mercury contamination, for instance, by using mercury-resistant bacteria (Fatimawali et al 2020).

Mercury poisoning in aquatic environments severely threatens the health and well-being of marine species, such as bacteria. While many bacteria are sensitive to the harmful effects of mercury, some have developed resistance mechanisms that enable them to live and even thrive in mercury-contaminated environments. Bioremediation can improve aquatic ecosystem health and resource sustainability by reducing mercury levels (Barkay & Wagner-Döbler 2005).

PT Genetika Science Indonesia accomplished the sequencing. With the Bioedit software, the sequences were edited and proofread. The MEGA 6.0 programme was used to build the phylogenetic tree using the maximum likelihood method and 1000 bootstrap iterations.

Genetic characteristics of bacteria from the coastal waters of Manado Bay, Totok Bay, and Likupang waters were confirmed using genetic identification. Electrophoresis of water containing mitochondrial DNA cytochrome oxidase 1 (CO1) genes, with amplicon lengths of approximately 1492 bp was conducted. Manado Bay (M1.2) water samples isolates were identified as *Pseudomonas hibiscicola*, Totok Bay (T1.5) water samples isolates were recognized as *Elizabethkingia anophelis* and Likupang (T1.4) water samples isolates were identified as *Acinetobacter pittii* (Table 4). The samples' of mitochondrial DNA CO1 gene nucleotide sequence alignment was associated to other NCBI information (NCBI 2023).

Table 4

BLAST results for water isolates

No	*Isolate code*	*BLAST results*
1	M1.2	*Pseudomonas hibiscicola*
2	T1.5	*Elizabethkingia anophelis*
3	T1.4	*Acinetobacter pittii*

BLAST (NCBI 2023) was utilized to identify the bacterial species isolated from coastal waters (see also Niane et al 2019). The results indicate that the three bacterial isolates belong to distinct species. *Pseudomonas hibiscicola*, *Elizabethkingia anophelis*, and *Acinetobacter pittii* were identified from isolate codes M1.2, T1.5, and T1.4. These results provide essential information regarding the bacterial diversity in coastal waters (Proenca et al 2022; Shahinyan et al 2017). Along with that, these findings provide valuable insight into the bacterial diversity prevalent in coastal waters. The bacterial strains isolated from coastal water could be used in biological wastewater treatment procedures and as aquafeed additives (Kim et al 2021). The study of bacterial populations inhabiting the sea surface microlayer at two contrasting Mediterranean coastal stations revealed that pollution levels affect bacterial diversity in coastal waters (Agogué et al 2005). BLAST determines the statistical significance of matches between nucleotide or protein sequences. It can be used to determine the members of a gene family as well as infer functional and evolutionary links between sequences. BLASTx, translated nucleotide sequence searched against protein sequences, was used to identify similarities between the sequences obtained and known sequences during the metagenomic analysis of a complex community found in pond detritus (Negi & Lal 2017).

Table 5

Results of proofreading the forward and reserve sample sequences M1.2, T1.5, and T1.4

Sample code	*Proofreading results*
M1.2	GGTTACCTTGTTACGACTTCACCCCAGTCATCGGCCACACCGTGGCAAGCGCCCCCCCGAAGGTTAA GCTACCTGCTTCTGGTGCAACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGT ATTCACCGCAGCAATGCTGATCTGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTC CAATCCGGACTGAGATAGGGTTTCTGGGATTGGCTTACCGTCGCCGGCTTGCAGCCCTCTGTCCCTAC CATTGTAGTACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCC GGTTTGTCACCGGCGGTCTCCTTAGAGTTCCCACCATTACGTGCTGGCAACTAAGGACAAGGGTTGC GCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTT CGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCGACATGTCAAGGCCAGGTAAGGTTCTTC GCGTTGCATCGAATTAAACCACATACTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCA GTCTTGCGACCGTACTCCCCAGGCGGCGAACTTAACGCGTTAGCTTCGATACTGCGTGCCAAATTGCA CCCAACATCCAGTTCGCATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACG CTTTCGTGCCTCAGTGTCAGTGTTGGTCCAGGTAGCTGCCTTCGCCATGGATGTTCCTCCTGATCTCT ACGCATTTCACTGCTACACCAGGAATTCCGCTACCCCCTACCACACTCTAGTCGCCCAGTATCCACTG CAGTTCACAGATTGAGCCCAGGGCTTTCACAACGGACTTAAACGACCACCTACGCACGCTTTACGCCC AGTAATTCCGAGTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGTGCTT ATTCTTTGGGTACCGTCATCCCAACCGGGTATTAGCCAGCTGGATTTCTTTCCCAACAAAAGGGCTTTA CAACCCGAAGGCCTTCTTCACCCACGCGGTATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCC CCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGATCATCCTCTCAGAC CAGCTACGGATCGTCGCCTTGGTGGGCCTTTACCCCGCCAACTAGCTAATCCGACATCGGCTCATTCA ATCGCGCAAGGCCCGAAGGTCCCCTGCTTTCACCCGTAGGTCGTATGCGGTATTAGCGTAAGTTTCCC TACGTTATCCCCCACGACAGAGTAGATTCCGATGTATTCCTCACCCGTCCGCCACTCGCCACCCAGAG

	AGCAAGCTCTCCTGTGCTGCCGTTCGACTTGCATGTGTTAGGCCTACCGCCAGCGTTCACTCTGAGCC ATGATCAAACTCTA
T1.5	GGTTACCTTGTTACGACTTAGCCCTAGTTACTAGTTTTACCCTAGGCAGCTCCTTTTACGGTCACCGAC TTCAGGTACCCCCAGCTTCCATGGCTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGC GCCATGGCTGATGCGCGATTACTAGCGATTCCAGCTTCATAGAGTCGAGTTGCAGACTCCAATCCGAA CTGAGACCGGCTTTCGAGATTTGCATCCTATCACTAGGTAGCTGCCCTCTGTACCGGCCATTGTAGCA CGTGTGTGGCCCAAGACGTAAGGGCCGTGATGATTTGACGTCATCCCCACCTTCCTCTCTACTTGCGT AGGCAGTCTCACTAGAGTCCTCAACTTAATGTTAGCAACTAGTGACAGGGGTTGCGCTCGTTGCAGGA CTTAACCTAACACCTCACGGCACGAGCTGACGACAACCATGCAGCACCTTGAAAAATGTCCGAAGAAG GATCTATTTCTAAATCTGTCATTTCCCATTTAAGTCTTGGTAAGGTTCCTCGCGTATCATCGAATTAAAC CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCATTCTTGCGAACGTACTCCC CAGGTGGATTACTTATCACTTTCGCTTAGTCTCTGAATCATTAAACCCAAAAACGAGTAATCATCGTTT ACGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGTCCATCAGCGTCAGTTAA GACTTAGTGACCTGCCTTCGCAATTGGTGTTCTAAGTAATATCTATGCATTTCACCGCTACACTACTTA TTCCAGCCACTTCAACCTTACTCAAGNGTGACAGTATCAATGGCAGTTCGACAGTAAGCTGTCGGATT TCACACTGACTTATCAGTCCGCCTACTGACCCTTAAACCCATTAATCCGGATAAGCCT*
T1.4	GGTTACCTTGTTACGACTTCACCCCAGTCATCGGCCACACCGTGGTAACCGCCCTCTTTGCAGTTAGG CTAGCTACTTCTGGTGCAACAAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTA TTCACCGCGGCATTCTGATCCGCGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCA ATCCGGACTACGATCGGCTTTTTGAGATTAGCATCCTATCGCTAGGTAGCAACCCTTTGTACCGACCA TTGTAGCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCGTCCCCGCCTTCCTCCA GTTTGTCACTGGCAGTATCCTTAAAGTTCCCGACATTACTCGCTGGCAAATAAGGAAAAGGGTTGCGC TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTATGTAA GTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTTACTATGTCAAGGCCAGGTAAGGTTCTTCGCG TTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAGTCT TGCGACCGTACTCCCCAGGCGGTCTACTTATCGCGTTAGCTGCGCCACTAAAGCCTCAAAGGCCCCAA CGGCTAGTAGACATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCATGCTTTC GCACCTCAGCGTCAGTGTTAGGCCAGATGGCTGCCTTCGCCATCGGTATTCCTCCAGATCTCTACGCA TTTCACCGCTACACCTGGAATTCTACCATCCTCTACCACACTCTAGCTAACCAGTATCGAAAGCAATTC CCAAGATAAGCTCGGGGATTTCACATTTGACTTAATTAGCCGCCTACGCGCGCTTTACGCCCAGTAAA TCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGCTTATTCTG CGAGTAACGTCCACTATCTCTAGGTATTAACTAAAGTAGCCTCCTCCTCGCTTAAAGTGCTTTACAACC ATAAGGCCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACT GCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCGGATCATCCCCTCAGACCCGC TACAGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACTTAGGCTCATCTATTAGC GCAAGGTCCGAAGATCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAGCATTCCTTTCGAAATGT TGTCCCCCACTAATAGGCAGATTCCTAAGCATTACTCACCCGTCCGCCGCTAAGATCAGTAGCAAGCT ACCTCTCTCCGCTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCAATCTGAGCCATGATCAAACT CTA

The results show that the sampled coastal water isolates belong to genetically distinct species. Table 5 displays the results of proofreading sequences in advance and in reverse as well as the sequences of the three isolates (M1.2, T1.5, and T1.4).

Table 6

Nucleotide sequence identification through BLAST analysis

Sample code	*Species outcome*	*BLAST*			
		Access code of NCBI	*Query cover (%)*	*E-value*	*Identity value (%)*
M1.2	*Pseudomonas hibiscicola*	JQ291604.1	99	0.0	99.67
T1.5	*Elizabethkingia anophelis*	NR_116021.1	99	0.0	99.05
T1.4	*Acinetobacter pittii*	CP049806.1	99	0.0	99.73

BLAST analysis was utilized to identify the nucleotide sequences supplied in the question. Using BLAST, the sample codes M1.2, T1.5, and T1.4 were compared to sequence databases, and the species results, NCBI access codes, query cover (%), E-value, and identity value (%) were obtained (Table 6).

According to the results, M1.2 is identified as *Pseudomonas hibiscicola*, T1.5 as *Elizabethkingia anophelis*, and T1.4 as *Acinetobacter pittii*.

The percentage of the query sequence that is covered by the subject sequence is indicated by the query cover. The E-value is the expected number of matches that would occur by coincidence in a database of the searched size. The identity value (percent) represents the proportion of identical amino acids or nucleotides shared by the query and subject sequences (Berkeley Library 2023; NCBI 2023).

These DNA sequences are pretty similar to the present sequences in the GenBank, according to the findings of the BLAST analysis (NCBI 2023). When the E-value is below 0.4, the sequence is highly comparable (Claverie & Notredame 2007).

Further analysis was carried out using phylogenetic trees to show the relationship between the samples (M1.2, T1.5 and T1.4) and several species (Figure 2, 3 and 4) available in the NCBI database (NCBI 2023). Genetic analysis illustrated that the samples were the same species.

Pseudomonas hibiscicola has been studied for its resistance to heavy metals and antibiotics in various environments, including coastal waters. According to the search results, *Pseudomonas* species isolated from sludge and effluent in Iraq, including *Pseudomonas hibiscicola*, were tested for their resistance to heavy metals (Alfarras et al 2022). Antibiotic and heavy metal resistance in gram-negative bacteria were investigated at Turkey's Seyhan Dam Lake and Seyhan River, and *Pseudomonas hibiscicola* was one of the species isolated (Matyar et al 2014). *Pseudomonas hibiscicola* was identified as one of the antibiotic-resistant and heavy metal-tolerant bacteria prevalent in clinical effluents and water samples (Nath et al 2020). *Pseudomonas hibiscicola* was identified as a bacterial species whose genes encode resistance to antibiotics and metals in Finland's well-maintained potable water distribution systems (Tiwari et al 2022). These studies indicate that *Pseudomonas hibiscicola* is resistant to heavy metals and antibiotics in various surroundings, as well as coastal waters (Alfarras et al 2022; Nguyen et al 2019).

Elizabethkingia anophelis has potential bioremediation applications due to its shared capacity with other bacteria to transform toxic mercury into less harmful forms (Chen et al 2019b; Nguyen et al 2019; Zajmi et al 2022). In addition, this bacterium has been associated with several ailments, especially in immunocompromised persons. Moreover, its mercury resistance may contribute to its antibiotic resistance (Cardona et al 2022; Zajmi et al 2022). *Elizabethkingia anophelis* is a mercury-resistant bacterium capable of surviving in sediments with increased mercury levels. Its resistance is probably due to a mercury resistance operon in its genome, which encodes essential mercury detoxification proteins. According to a study, there is a connection between antibiotic resistance and bacterial resistance to heavy metals (Chen et al 2019a; Lau et al 2016; Nguyen et al 2019; Vignaroli et al 2018).

Numerous strains of *Acinetobacter* exhibit extensive drug resistance, and some members of the genus play a crucial role in removing heavy metals from polluted environments (Domingues et al 2021). *Acinetobacter pittii* was identified as 255 gram-negative bacterial species whose resistance to 16 antibiotics and five heavy metals was investigated. *Citrobacter koseri*, *Escherichia coli*, and *Pantoea agglomerans* were the most typical isolates isolated from all samples (Matyar 2012). *Acinetobacter pittii* has been considered for its resistance to heavy metals and antibiotics in various environments (Chaturvedi et al 2021; Li et al 2017).

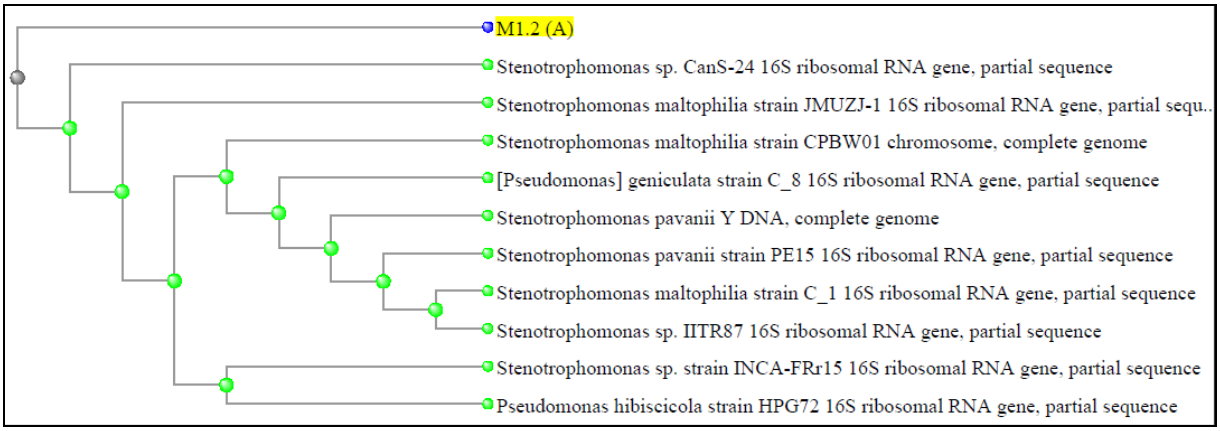

Figure 2. Phylogenetic analysis for *Pseudomonas hibiscicola* (generated using MEGA 11 software).

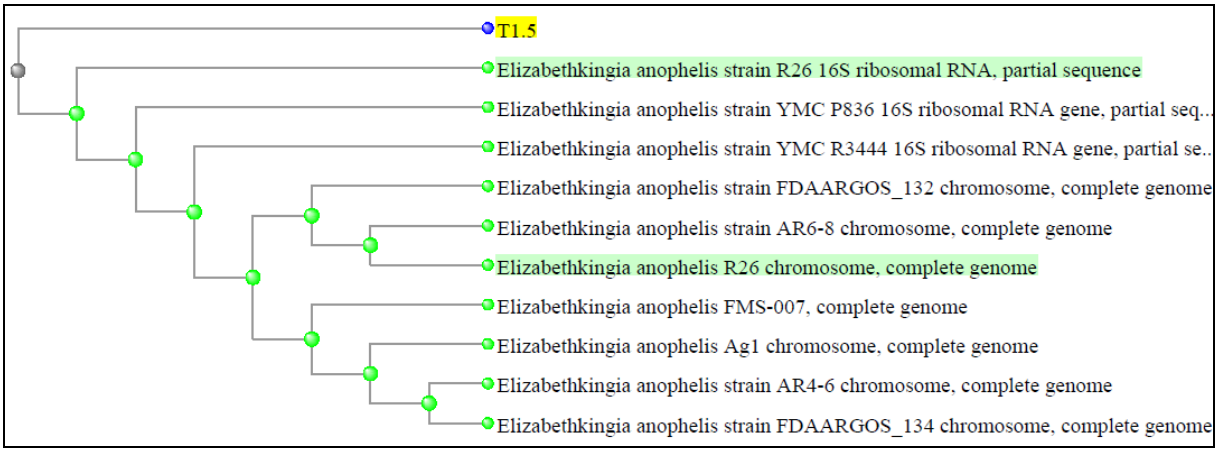

Figure 3. Phylogenetic analysis for *Elizabethkingia anophelis* (generated using MEGA 11 software).

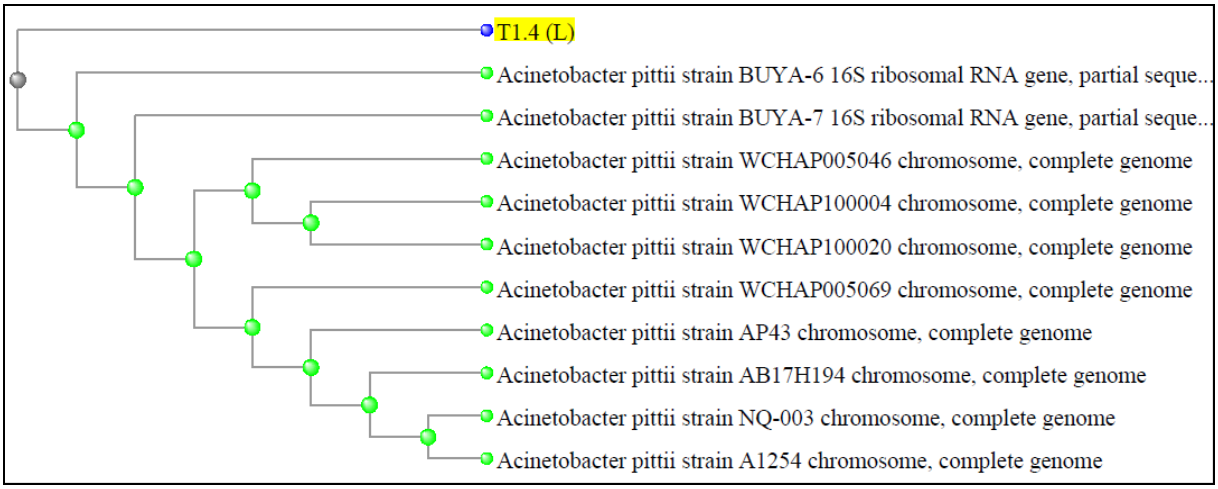

Figure 4. Phylogenetic analysis for *Acinetobacter pittii* (generated using MEGA 11 software).

Conclusions. The presence of antibiotic-resistant and heavy metal-tolerant bacteria in water sources is a public health concern because it can result in the dissemination of genes associated with antibiotic resistance and the environmental survival of these microorganisms.

Due to the potential ecological and health effects of mercury toxicity, mercury-resistant bacteria presence in coastal waters is exceptionally concerning. According to the study's findings, the mercury-resistant bacteria isolated from North Sulawesi's coastal waters are *Pseudomonas hibiscicola*, *Elizabethkingia anophelis*, and *Acinetobacter pittii*. More research is required to assess the effects of these bacteria on the ecosystem and better comprehend their diversity, distribution, and function in coastal waters. The prevalence of mercury-resistant bacteria in littoral waters is worrisome because mercury poisoning can negatively affect ecosystems and human health. Mercury poisoning demonstrates the need for conservation efforts in coastal regions to reduce mercury contamination and protect the ecosystem and its inhabitant's health. Proper mercury pollution control may affect aquatic ecology and resource conservation. Bioremediation may improve aquatic ecosystem health and resource sustainability by reducing mercury levels.

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