COASTAL WATERS UNDER THE MICROSCOPE: SPATIAL CONTAMINATION AND ANTIMICROBIAL RESISTANCE IN BARRA, TUDELA

A RESEARCH PAPER

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In Partial Fulfillment
of the Requirements for the Degree
BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY

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June 2026

Misamis University

Ozamiz City
COLLEGE OF ARTS AND SCIENCES

DEPARTMENT OF NATURAL SCIENCES

CERTIFICATE OF PANEL APPROVAL

The research paper attached hereto, entitled **"COASTAL WATERS UNDER THE MICROSCOPE: SPATIAL CONTAMINATION AND ANTIMICROBIAL RESISTANCE IN BARRA, TUDELA"**, prepared and submitted by **FEBIE JOY R. BUHANGIN, ZYVIL PRINCESS S. CABASIS, CARBIE O. SIMBAJON, AND DOREEN D. TACTACON**, in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, is hereby recommended for approval.

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ABSTRACT

Coastal waters are increasingly threatened by microbial contamination and antimicrobial resistance resulting from anthropogenic activities and inadequate sanitation practices. This study investigated the occurrence of *Escherichia coli* and *Vibrio* spp. and evaluated their antimicrobial susceptibility profiles in the coastal waters of Barangay Barra, Tudela, Misamis Occidental. Water samples were collected monthly from February to April 2026 across nine sampling stations located along the shoreline, 50 m offshore, and 100 m offshore. Standard microbiological techniques were employed for bacterial isolation and identification, while antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method. Results demonstrated markedly higher bacterial contamination in nearshore stations, where *E. coli* counts were recorded as Too Numerous To Count (TNTC), with bacterial density progressively decreasing offshore. *Vibrio* spp. counts ranged from 55 ± 18.7 to 159 ± 15.0 CFU/mL, corresponding to 6.72 ± 0.17 to 7.20 ± 0.04 $\log_{10}$ CFU/mL. Significant spatial differences in microbial contamination were observed among sampling stations (Kruskal–Wallis: $H = 36.12$, $p = 8.4 \times 10^{-6}$ for EMB counts; $H = 38.53$, $p = 6.01 \times 10^{-6}$ for *Vibrio* spp.). Antimicrobial susceptibility testing revealed high resistance to ampicillin in both bacterial groups, whereas ciprofloxacin demonstrated the greatest antibacterial activity against the isolates. The findings indicate persistent microbial contamination and the presence of antibiotic-resistant bacteria in the coastal waters of Barangay Barra, emphasizing the need for continuous microbial surveillance, improved sanitation practices, and strengthened coastal environmental management to protect public health.

Keywords: *antimicrobial resistance, antimicrobial susceptibility testing, coastal water quality, Escherichia coli, Vibrio spp.*

DEDICATION

This paper is dedicated to our families, friends, and loved ones,
whose endless support, encouragement, and understanding
became our strength throughout this journey.

And also,

To the people of Barra, whose community, culture, and environment
inspired this study and encouraged us to create meaningful research
for positive change.

And,

To our Mother Earth,
the source of life and beauty,
may we continue to protect and
preserve nature for future generations.

The Researchers

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

INTRODUCTION

Background of the Study

Coastal waters are among the most productive and ecologically significant environments in the world, providing essential resources for fisheries, transportation, recreation, tourism, and coastal livelihoods. Despite occupying less than 10% of the global ocean area, coastal ecosystems contribute substantially to marine biodiversity, nutrient cycling, and primary productivity. However, rapid urbanization, population growth, industrialization, and increasing anthropogenic activities have intensified the degradation of coastal water quality worldwide. Recent reports have shown that untreated domestic wastewater, agricultural runoff, plastic pollution, and improper waste disposal continue to introduce large amounts of contaminants into marine ecosystems, particularly in developing countries where sanitation infrastructure remains inadequate (UNEP, 2023; WHO, 2022).

Microbial contamination is one of the major environmental and public health concerns affecting coastal waters. Contaminated coastal environments serve as reservoirs of pathogenic microorganisms that may threaten human health through recreational exposure, seafood consumption, fishing activities, and direct contact with polluted water. According to the Environmental Health Theory, environmental conditions such as poor sanitation, contaminated water systems, and improper waste management directly influence disease transmission and community health outcomes. Coastal communities that rely heavily on marine resources are therefore highly vulnerable to waterborne diseases associated with microbial pollution.

Among the most significant waterborne bacterial pathogens found in marine and estuarine environments are *Vibrio* species. *Vibrio* spp. are Gram-negative, rod-shaped bacteria naturally occurring in coastal and brackish waters, with several species recognized as important human pathogens. Recent studies have reported increasing occurrences of *Vibrio*-associated infections linked to climate change, warming coastal waters, nutrient enrichment, and environmental pollution (Baker-Austin et al., 2023; Fu et al., 2022). Human infection commonly occurs through the consumption of contaminated seafood or exposure to contaminated seawater, leading to illnesses ranging from gastroenteritis to wound infections and septicemia. Environmental factors such as elevated temperature, increased organic matter, and poor sanitation practices further enhance the survival and proliferation of *Vibrio* spp. in coastal ecosystems.

Another important microbial indicator of water quality is *Escherichia coli*, a facultative anaerobic Gram-negative bacterium commonly found in the intestinal tract of humans and warm-blooded animals. The presence of *E. coli* in aquatic environments is widely recognized as an indicator of fecal contamination and the possible presence of pathogenic microorganisms originating from sewage or animal waste. Monitoring *E. coli* in recreational and coastal waters is essential because elevated concentrations are strongly associated with increased risks of gastrointestinal illnesses and other waterborne infections (WHO, 2023). Recent studies have identified untreated sewage discharge, stormwater runoff, septic tank leakage, and anthropogenic coastal activities as major contributors to *E. coli* contamination in marine environments (Ahmed et al., 2022; Perkins et al., 2023).

The Fecal Indicator Bacteria Theory supports the use of *E. coli* as an indicator organism for assessing microbial water quality. This theory explains that detecting fecal

indicator bacteria reflects potential contamination by pathogenic microorganisms derived from human or animal feces. Since direct detection of all pathogens in aquatic systems is often difficult and expensive, indicator organisms such as *E. coli* are widely used to evaluate sanitary conditions and public health risks in coastal environments. In recent years, the emergence of antimicrobial resistance (AMR) among environmental bacterial isolates has become a growing global health concern. Coastal waters may act as reservoirs and transmission pathways for antibiotic-resistant bacteria because they receive wastewater containing antibiotic residues, resistant microorganisms, and other environmental contaminants. Environmental isolates of *E. coli* and *Vibrio* spp. have increasingly demonstrated resistance to commonly used antibiotics, including penicillins, cephalosporins, and quinolones (Mughini-Gras et al., 2023; Murray et al., 2022). The spread of antimicrobial-resistant bacteria in aquatic ecosystems poses serious risks because resistance genes may potentially transfer to clinically important pathogens, thereby reducing treatment effectiveness and complicating disease management.

The Theory of Selective Pressure in Antimicrobial Resistance explains that repeated exposure of microorganisms to antimicrobial compounds promotes the survival and proliferation of resistant bacterial strains. Antibiotic residues introduced into aquatic environments through domestic wastewater, hospital discharge, aquaculture, and agricultural runoff create selective pressure that accelerates the development and spread of resistance genes among bacterial populations. Consequently, antimicrobial susceptibility testing has become essential in identifying effective antibiotics, monitoring resistance trends, and assessing potential environmental and public health risks associated with contaminated water systems.

Barangay Barra is a coastal barangay located in the municipality of Tudela, Misamis Occidental, with a population of 1,415 based on the 2020 Census of the Philippine Statistics Authority. The community relies heavily on marine and coastal resources for fishing, livelihood, transportation, tourism-related activities, and daily subsistence. Due to its proximity to coastal waters, the environmental condition of the area directly affects community health, sanitation, and economic stability. Similar to many coastal communities in the Philippines, Barangay Barra remains vulnerable to environmental challenges such as improper waste disposal, domestic wastewater discharge, inadequate sanitation infrastructure, and contamination of coastal waters.

For several years, Barangay Barra has served as one of the adopted communities of Misamis University through various extension and outreach programs. The College of Maritime Education has actively conducted environmental initiatives including coastal clean-up drives, mangrove planting activities, sanitation-related projects, and environmental awareness campaigns aimed at promoting environmental stewardship and improving community sanitation practices.

The Community-Based Environmental Management Theory emphasizes the importance of collaborative participation among academic institutions, local communities, and government agencies in addressing environmental problems. According to this theory, community participation and environmental education contribute significantly to the success of environmental conservation and sanitation programs. Despite the implementation of these environmental initiatives, limited scientific studies have evaluated their effectiveness in reducing microbial contamination and improving coastal water quality in Barangay Barra.

Moreover, there remains a lack of localized scientific data regarding the occurrence of *Escherichia coli*, *Vibrio* spp., and antimicrobial resistance patterns in the coastal waters of Barra, Tudela, Misamis Occidental. While physical improvements in environmental cleanliness may be observed, harmful microorganisms may persist in coastal waters exposed to continuous anthropogenic activities and wastewater contamination. The absence of baseline microbial and antimicrobial resistance data limits the ability of local government units and environmental agencies to develop evidence-based sanitation and coastal management strategies.

Therefore, this study was conducted to investigate the occurrence of *Escherichia coli* and *Vibrio* spp. and determine their antimicrobial susceptibility profiles in the coastal waters of Barangay Barra, Tudela, Misamis Occidental.

Objectives of the Study

This study aimed to assess the microbial contamination and antimicrobial susceptibility profiles of *Escherichia coli* and *Vibrio* spp. in the coastal waters of Barangay Barra, Tudela, Misamis Occidental. Specifically, this study:

1. Determined the presence and concentration of *Escherichia coli* in the coastal waters of Barangay Barra across different sampling stations;
2. Determined the presence and concentration of *Vibrio* spp. in the coastal waters of Barangay Barra across different sampling stations;
3. Compared the spatial distribution of *E. coli* and *Vibrio* spp. among shoreline, 50-meter offshore, and 100-meter offshore sampling stations;

4. Determined the antimicrobial susceptibility profiles of the isolated *Escherichia coli* and *Vibrio* spp. using selected antibiotics through the Kirby–Bauer disk diffusion method;
5. Identified the most effective antibiotics against the isolated bacterial strains based on susceptibility patterns; and
6. Evaluated the potential public health implications of microbial contamination and antimicrobial resistance in the coastal waters of Barangay Barra.

Significance of the Study

This study is significant as it provides scientific evidence on the microbial contamination and antimicrobial susceptibility of *Escherichia coli* and *Vibrio* spp. in the coastal waters of Barangay Barra, Tudela, Misamis Occidental. The findings will benefit the local community by increasing awareness of potential health risks associated with exposure to contaminated coastal waters and encouraging improved sanitation and environmental practices. For the Local Government Unit of Tudela, the results may serve as a basis for strengthening coastal water quality monitoring, sanitation policies, and environmental protection programs aimed at reducing microbial pollution. As Barangay Barra is an adopted community of Misamis University, the study may also help evaluate the effectiveness of existing outreach and environmental initiatives, thereby guiding future extension activities focused on coastal sanitation and public health. Furthermore, the study provides baseline data that may assist environmental and public health agencies in assessing contamination levels, identifying potential risks, and implementing appropriate intervention measures. It also serves as a useful reference for future researchers in

environmental microbiology, antimicrobial resistance, and coastal water quality assessment, particularly in similar coastal settings. Overall, the study contributes to the broader understanding of microbial pollution and antimicrobial resistance in coastal environments and highlights its implications for environmental management and public health protection.

Scope and Delimitations

This study was conducted in Purok-3, Barangay Barra, Tudela, Misamis Occidental. It focused on the detection and analysis of bacterial organisms, specifically *Escherichia coli* and *Vibrio* spp., as indicators of microbial water quality and potential public health risk. Laboratory analyses were limited to microbial isolation, identification, and antimicrobial susceptibility testing using standard bacteriological methods. Sampling was carried out over three consecutive months—February, March, and April 2026- to assess bacterial levels and water quality conditions within this specific period.

This study did not include the detection of other pathogens such as viruses, parasites, or protozoa, nor did it assess chemical or physical pollutants that may also influence overall water quality. Furthermore, the findings are confined to the selected sampling sites and the specific timeframe of the study; thus, they may not be generalized to other coastal areas within Tudela or neighboring municipalities.

MATERIALS AND METHODS

Research Design

The study utilized a descriptive-experimental research design that integrated laboratory-based experiments and quantitative analysis. This design combined quantitative microbiological analyses, such as antibiotic susceptibility testing, with qualitative field observations, allowing a comprehensive evaluation of marine water quality (Jeamsripong et al., 2022). Comparative analyses were conducted across different sampling sites and distances (Bărbulescu & Barbeș, 2026). The study aimed to detect, quantify, and assess the antibiotic resistance of *Escherichia coli* and *Vibrio spp*. isolated from the coastal waters of Barangay Barra, Tudela, Misamis Occidental.

Research Setting

The study was conducted in Purok-3, Barangay Barra, Tudela, Misamis Occidental (8.247722, 123.855620), a coastal community characterized by residential households and nearby small-scale livestock facilities, including piggeries and septic systems located near the shoreline. These environmental conditions make the area potentially vulnerable to coastal water contamination from anthropogenic sources. Barangay Barra is also an adopted community of Misamis University, which makes it a suitable site for community-based research and environmental monitoring initiatives. Figure 1 shows the map of the coastal sampling sites established in Barangay Barra, Tudela, Misamis Occidental, including the nine sampling stations distributed across the shoreline, 50-meter offshore, and 100-meter offshore zones.

Coastal water sampling was carried out in Purok 3, where nine (9) sampling stations were established to represent different spatial points along the shoreline. Water samples were collected at three designated distances from the shore: nearshore (0 meters), 50 meters offshore, and 100 meters offshore. The nearshore or shoreline stations included Station 1 (8.24751, 123.85556), Station 2 (8.24755, 123.85554), and Station 3 (8.24759, 123.85553). The 50-meter offshore stations included Station 4 (8.24774, 123.85596), Station 5 (8.24771, 123.85594), and Station 6 (8.24781, 123.85591). Meanwhile, the 100-meter offshore stations included Station 7 (8.24797, 123.85635), Station 8 (8.24798, 123.85633), and Station 9 (8.24802, 123.85629). This sampling design allowed for the assessment of spatial variations in microbial contamination across different zones of coastal exposure.

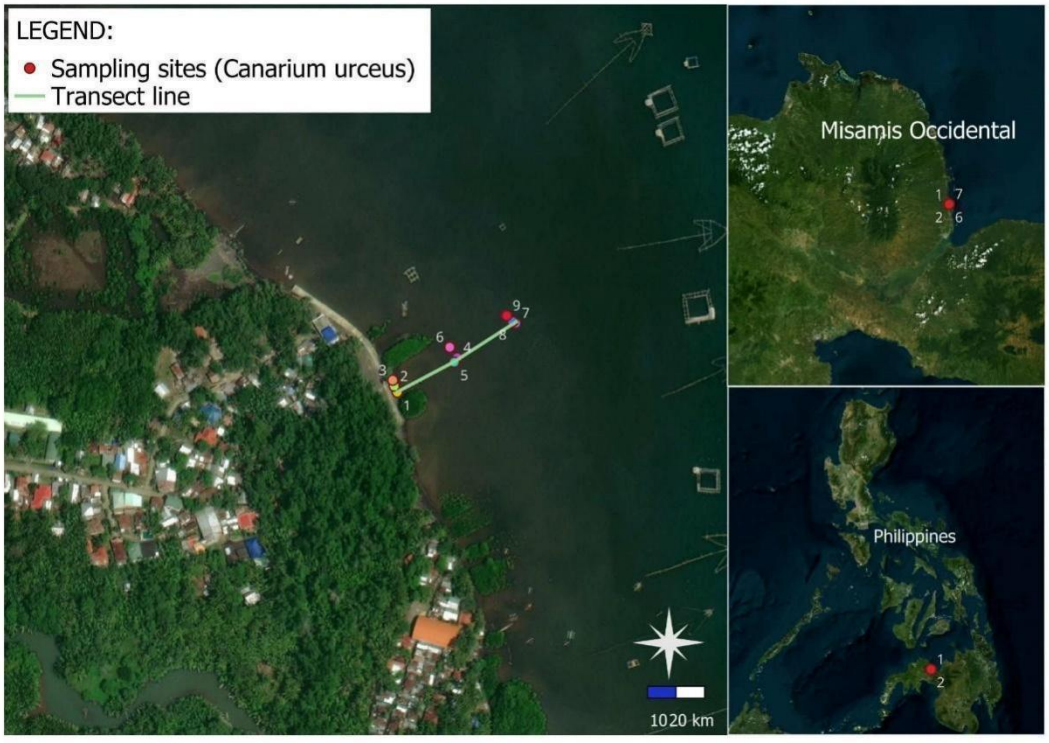

Figure 1. Map of Coastal Sampling Sites in Barra, Tudela, Misamis Occidental

Conceptual Framework

Figure 2 presents the methodological framework used to investigate the spatial distribution and antibiotic resistance of *Escherichia coli* and *Vibrio* spp. in the coastal waters of Barangay Barra, Tudela, Misamis Occidental. Water samples were collected from nine sampling stations located at three distances from the shoreline (nearshore, 50 m, and 100 m). A 500 mL volume of water was collected from each station under sterile conditions and transported in an icebox at approximately 4°C for laboratory processing within 6–8 hours. Community assessment through surveys, observations, and infrastructure mapping was also conducted to identify potential sources of contamination.

The collected samples were analyzed for the presence and concentration of *E. coli* and *Vibrio* spp. Detection of *E. coli* involved presumptive testing using Lauryl Sulfate Tryptose (LST) broth, followed by confirmatory testing using EC broth. Positive samples were further cultured on Eosin Methylene Blue (EMB) agar and subjected to Gram staining for verification. For *Vibrio* spp., samples were isolated and enumerated through spread-plating on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar.

Following bacterial isolation, colony counts were determined to assess contamination levels across the sampling stations. Representative isolates were then subjected to antibiotic susceptibility testing using the Kirby–Bauer disk diffusion method on Mueller–Hinton Agar (MHA). The resulting data were log-transformed and statistically analyzed using the Shapiro–Wilk test for normality, the Kruskal–Wallis test to determine significant differences among sampling stations, and Dunn's post hoc test for pairwise comparisons. The findings were used to evaluate the spatial distribution of bacterial

contamination and the antibiotic resistance patterns of *E. coli* and *Vibrio* spp. in the coastal waters of Barangay Barra.

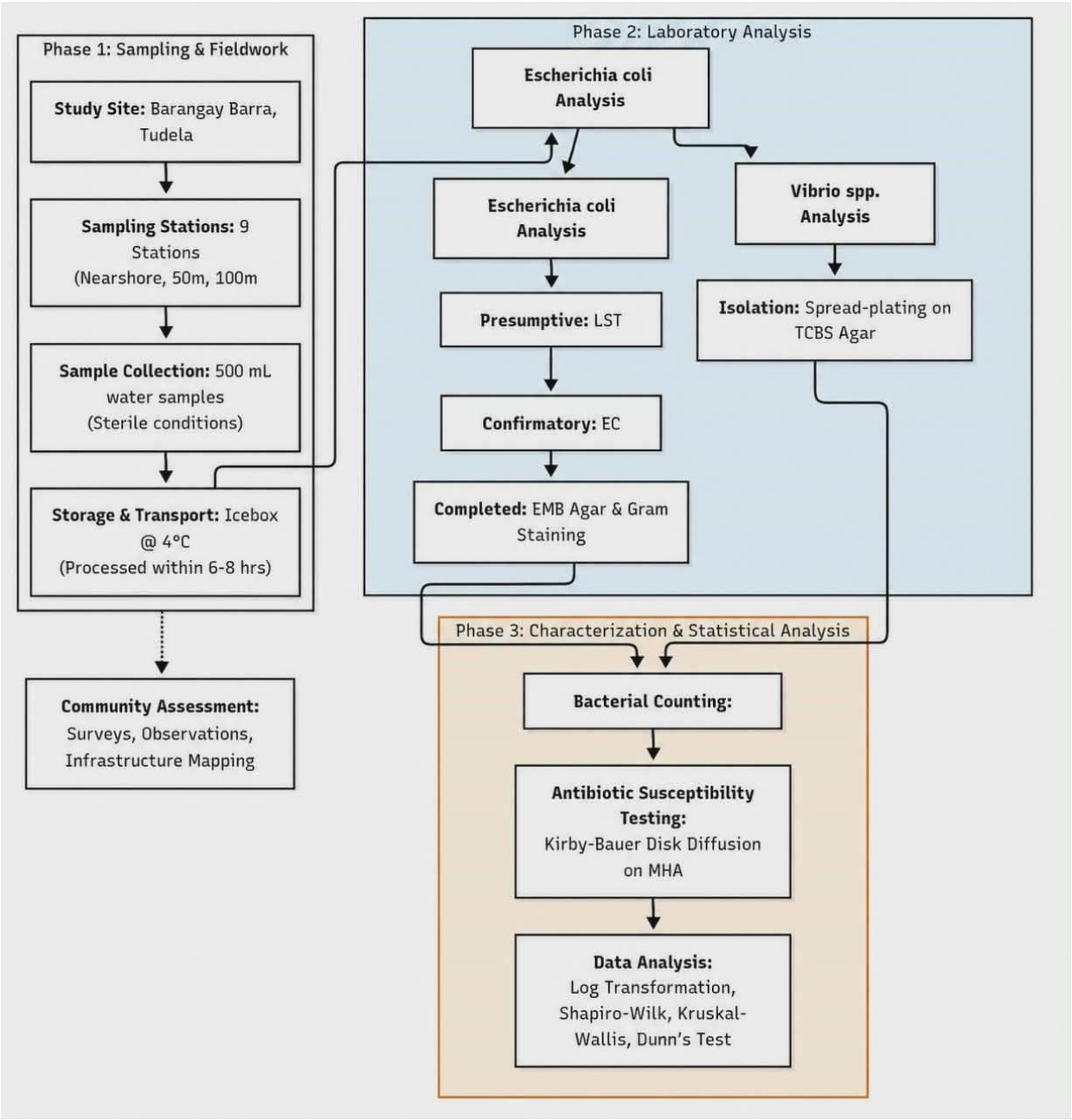

Figure 2. Schematic Diagram of the Study

Sample Collection, Storage, and Transport

To ensure the integrity of the water samples and the accuracy of the microbiological analysis, strict aseptic protocols were followed. Sampling was conducted during the dry season, specifically in February, March, and April, in the coastal waters of Barangay Barra, Tudela. In accordance with established procedures for assessing microbial contamination and spatial distribution in coastal areas (Bărbulescu & Barbeș, 2026), sampling stations were established in Purok 3, covering nine (9) sampling stations at three distances: the shoreline (near shore), 50 meters, and 100 meters offshore.

At each sampling point, 500 mL of water was collected using sterile glass containers. To prevent external contamination, researchers wore gloves throughout the process and used sterile instruments for all collection tasks. All tools and equipment, including iceboxes and containers, were thoroughly sterilized before and after each use. Immediately following collection, samples were stored in an icebox maintained at approximately 4 °C using ice packs to preserve microbial integrity during transit. Following APHA guidelines (2017), all samples were transported to the laboratory and processed within 6 to 8 hours to maintain bacterial viability and ensure accurate analysis of *Escherichia coli* and *Vibrio* spp. This rigorous handling and storage protocol ensured that the samples remained representative of the coastal environment for both isolation and antibiotic susceptibility testing.

Microbial Analysis of Water Samples

Microbial analyses were conducted at the Microbiology Laboratory, Maria Mercado Hall, and the Natural Science Laboratory of Misamis University, which provided the necessary equipment and sterile conditions for the isolation and identification of *Escherichia coli* and *Vibrio* spp.

Escherichia coli Analysis: Presumptive, Confirmatory, and Completed Tests

From each 500 mL water sample collected, a 50 mL aliquot was used for analysis and serially diluted using sterile normal saline solution (NSS) to prepare a 10^{-1} dilution to obtain a countable range of coliforms. Following the procedures outlined by the American Public Health Association (APHA, 2017), each sample was homogenized by shaking the container 25 times in a back-and-forth motion over a distance of approximately 30 cm within seven seconds to ensure even microbial distribution, as shown in Figure 3, which illustrates the Presumptive and Confirmatory Test for *Escherichia coli*.

Aliquots of 1 mL from each dilution were inoculated into triplicate tubes containing 9 mL Lauryl Sulfate Tryptose (LST) broth (final volume: 10 mL per tube) using sterile 1 mL pipettes. Inoculation was carefully performed by holding the pipette at an angle against the inner wall of the tube to minimize contamination and aerosol formation. Following the FDA Bacteriological Analytical Manual (FDA BAM, 2020), all dilution and inoculation procedures were completed within 15 minutes to preserve microbial viability.

The inoculated LST tubes were incubated at 35 °C ± 0.5 °C and observed for turbidity and gas production in Durham tubes. After 24 ± 2 hours, tubes were examined for evidence of gas formation, with final confirmation conducted at 48 ± 3 hours for negative

results (APHA, 2017). Tubes exhibiting both turbidity and gas production were recorded as presumptive positive for coliform bacteria and subjected to confirmatory testing.

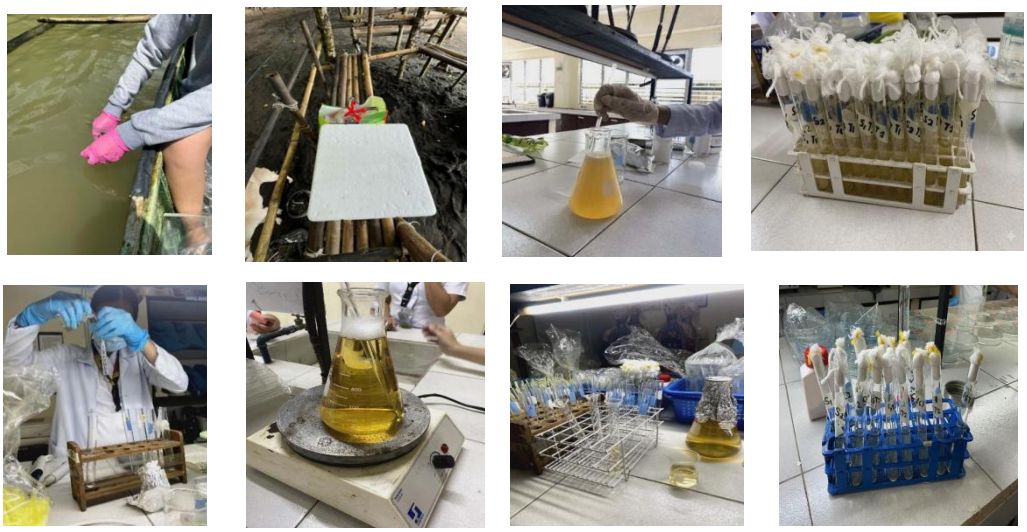

Figure 3. Presumptive and Confirmatory Test for Escherichia coli

For confirmatory analysis, a loopful from each gas-positive LST tube was inoculated into Escherichia coli (EC) broth and incubated at 45.5 °C for 24 ± 2 hours. Gas production was recorded as indicative of fecal coliform presence. Tubes negative after 24 hours were re-incubated and re-examined after 48 ± 2 hours.

For completed testing, a loopful from each gas-positive EC broth culture was streaked onto Eosin Methylene Blue (EMB) agar using the quadrant streak method to obtain isolated colonies. Plates were incubated inverted at 37 °C for 24–48 hours. This stage of analysis is further presented in Figure 4, which shows a completed test for *Escherichia coli* via Gram staining, demonstrating Gram-negative, rod-shaped morphology. Due to the unavailability of IMViC biochemical tests during the study period, bacterial identification was based on characteristic colony morphology on selective media,

Gram-negative reaction, and rod-shaped cellular structure. Samples exhibiting at least one isolate consistent with these criteria were considered positive for *E. coli* (APHA, 2017; Bhandari et al., 2007).

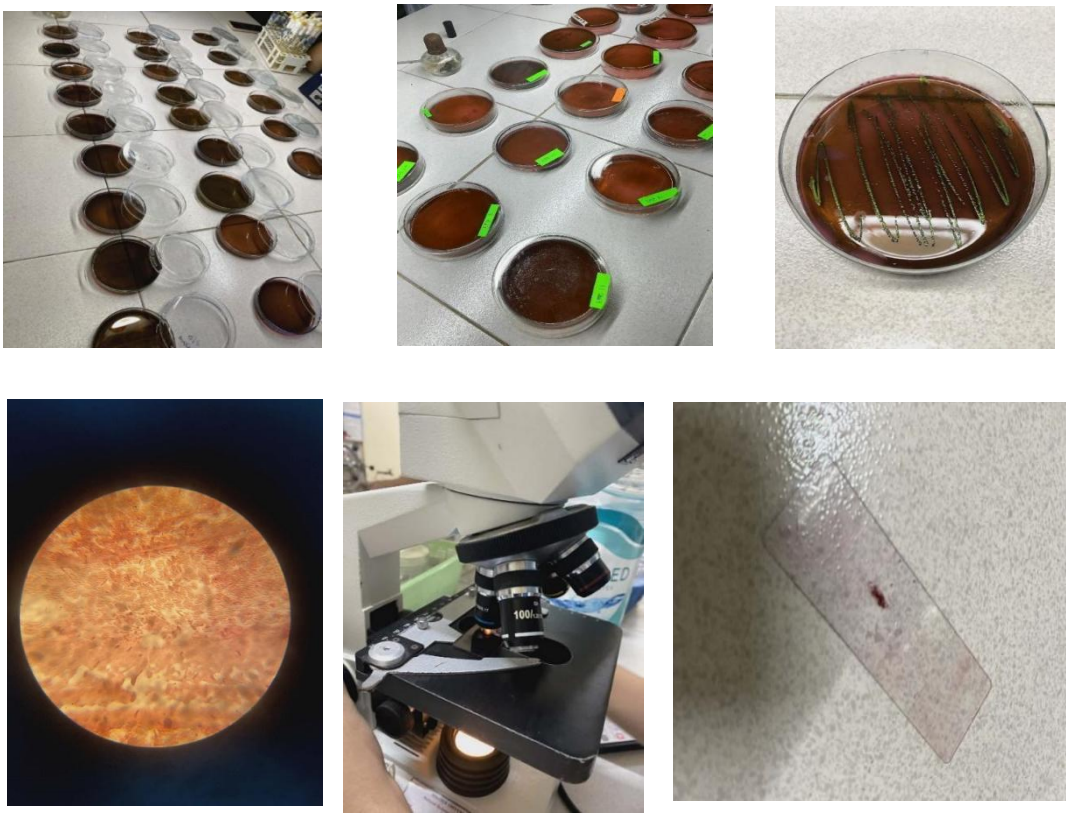

Figure 4. Completed Test for Escherichia coli via Gram staining showing Gram-negative, rod-shaped morphology.

Vibrio spp.: Isolation and Analysis

The 1 mL of water samples were serially diluted in 9 mL alkaline peptone water (APW), and 1 mL from the positive APW tubes was spread-plated onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar using the L-shaped glass rod method. The plates were incubated at 37 °C for 24 hours. Following incubation, presumptive *Vibrio* spp. Colonies

exhibiting shiny yellow, smooth, convex, slightly flattened surfaces, and opaque centers were enumerated for viable counts (Hosen et al., 2021), as shown in Figure 5, which illustrates *Vibrio* spp. on TCBS agar with characteristic yellow, sucrose-fermenting colonies.

Bacterial counts were expressed in colony-forming units per milliliter (CFU/mL), and representative isolates were recorded for further analysis. All procedures were conducted following standard microbiological practices to ensure accuracy, reliability, and prevention of cross-contamination.

Figure 5. Vibrio spp. on TCBS Agar exhibiting characteristic yellow, sucrose-fermenting colonies.

Antimicrobial Susceptibility Testing

The antibacterial susceptibility of the isolated microorganisms was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) (CLSI, 2026). A range of commonly used antibiotics, including ampicillin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and chloramphenicol (30 µg), were tested. To ensure a standardized inoculum, bacterial colonies were suspended in sterile saline until the turbidity matched a 0.5 McFarland standard. A dry, sterile swab was dipped into the inoculum tube. It was then streaked on the entire surface of the MHA plate. After plating, the antibiotic disks were placed on the agar surface within 15 minutes of inoculation using sterile forceps. The disks were placed 24mm apart to avoid overlapping zones. Once all disks were in place, the plates were incubated at 37 °C for 24 hours. After incubation, the diameters of the zones of inhibition around each disc were measured in millimeters using a vernier caliper as shown in Figure 6, which illustrates the antimicrobial susceptibility testing of *Escherichia coli* and *Vibrio* spp.

Interpretation of results was based on Clinical and Laboratory Standards Institute (CLSI, 2026) breakpoints as follows: ampicillin (S ≥ 17 mm, I = 14–16 mm, R ≤ 13 mm), ceftriaxone (S ≥ 23 mm, I = 20–22 mm, R ≤ 19 mm), ciprofloxacin (S ≥ 21 mm, I = 16–20 mm, R ≤ 15 mm), gentamicin (S ≥ 15 mm, I = 13–14 mm, R ≤ 12 mm), and chloramphenicol (S ≥ 18 mm, I = 13–17 mm, R ≤ 12 mm). Isolates were categorized as susceptible, intermediate, or resistant based on these zone diameter standards (CLSI, 2026).

Isolates that exhibited resistance to at least one antibiotic in three or more antimicrobial classes were classified as multidrug-resistant (MDR) (Magiorakos et al., 2012; Jeamsripong et al., 2022). Given the critical status of antimicrobial resistance in

marine ecosystems, monitoring these patterns is essential for assessing environmental health risks (WHO, 2024; Neetoo et al., 2026)

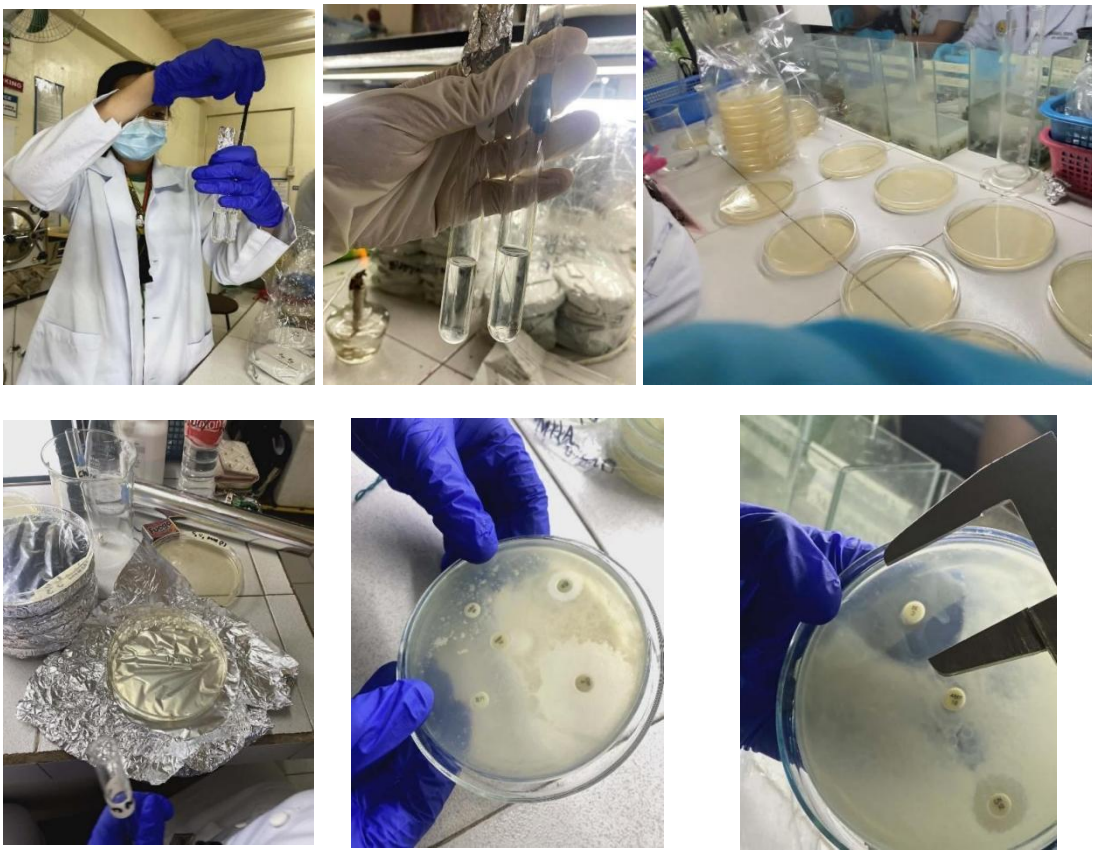

Figure 6. Antimicrobial Susceptibility Test E.coli and Vibrio spp.

Bacterial Count of *Escherichia coli* and *Vibrio spp.*

The bacterial load of the coastal water samples was quantified by determining the number of colony-forming units (CFU) per milliliter based on the growth observed on selective and differential culture media, as shown in Figure 7, which illustrates colony counting of bacterial growth. *Escherichia coli* was enumerated using Eosin Methylene Blue (EMB) agar, wherein characteristic colonies exhibiting a metallic green sheen were

considered indicative of strong lactose fermentation and presumptive identification of *E. coli*. In contrast, *Vibrio* spp. were enumerated using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar, a selective and differential medium specifically designed for the isolation of *Vibrio* species (Su & Liu, 2007). Colony counts were recorded and expressed as CFU/mL using standard microbiological enumeration procedures.

The computed bacterial counts served as indicators of water quality and potential contamination sources. Elevated levels of *E. coli* were interpreted as evidence of fecal contamination, typically associated with sewage discharge, stormwater runoff, or inadequate sanitation practices. Meanwhile, the presence of *Vibrio* spp. reflected natural marine bacterial populations that may proliferate under favorable environmental conditions and may also indicate anthropogenic influence in coastal waters. The detection of both organisms in recreational waters is of public health concern due to their association with waterborne diseases and gastrointestinal infections.

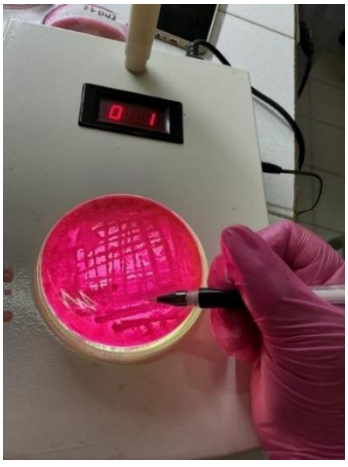

Figure 7. Colony Counting of Bacterial Growth

Community Assessment

A community assessment was conducted alongside the environmental survey to evaluate local sanitation methods and infrastructure. This included examining the condition and placement of piggeries, the construction of septic tanks, and overall sanitation facilities, following environmental health survey guidelines (Prüss-Ustün et al., 2019). The residents were interviewed to assess their awareness of waterborne diseases and sanitation practices (Creswell & Creswell, 2018). Community participation was considered essential for identifying and addressing environmental health issues (Corburn, 2005). Observations were documented through photographs, maps, and sketches to support the triangulation of results. As shown in Figure 8, anthropogenic infrastructure such as residential septic tanks and comfort rooms were observed near the high-tide mark, indicating a potential risk of fecal leaching into the coastal water. It also presents potential sources of fecal pollution, including stray animals and visible animal waste within the coastal collection area of Barangay Barra. Figure 9 shows the potential sources of fecal pollution of stray animals and animal waste within the coastal collection area of Barangay Barra. Figure 10 illustrates the accumulation of anthropogenic debris along the shoreline, including plastic and organic waste subjected to tidal movement and capable of harboring pathogenic microorganisms. Meanwhile, Figure 11 shows common human activities at the site, including children swimming in the water and fishing boats (bangkas) docked along the shoreline.

Figure 8. Anthropogenic Infrastructure: Documentation of residential septic tanks and comfort rooms adjacent to the high-tide mark, indicating risks of fecal leaching.

Figure 9. Potential Sources of Fecal Pollution: Documentation of stray animals and animal waste within the coastal collection area of Barangay Barra.

Figure 10: Accumulation of Anthropogenic Debris: Plastic and Organic Waste Along the Shoreline, Subject to Tidal Wash, and Capable of Harboring Pathogenic Microorganisms

Figure 11. Common Activities at the Site: Children swimming in the water and fishing boats (bangkas) parked along the shore.

Data Analysis

Quantitative and descriptive data were obtained through microbial isolation, colony enumeration, biochemical characterization, and antimicrobial susceptibility profiling to assess the contamination levels of *Escherichia coli* and *Vibrio* spp. in the coastal waters of Barangay Barra, Tudela, Misamis Occidental.

To determine microbial load, colony counts observed on culture plates were used to compute colony-forming units per milliliter (CFU/mL). The average colony count was calculated and expressed using the standard formula (Dumaloan-Canini et al., 2024):

$$CFU/mL = \frac{Number\ of\ colonies\ \times Dilution\ factor}{Volume\ of\ culture\ plated\ (mL)} \quad (1)$$

To normalize data distribution and facilitate statistical analysis, CFU/mL values were transformed into logarithmic form (log_{10} CFU/mL) using the standard expression (Dumaloan-Canini et al., 2024):

$$Log\frac{CFU}{ml} = Log_{10}(CFU/mL) \quad (2)$$

For antimicrobial susceptibility testing, the diameters of zones of inhibition (in millimeters) were measured for each antibiotic disc. These measurements served as the basis for determining the response of bacterial isolates to each antimicrobial agent. The percentage resistance for each antibiotic was computed using the formula (Dumaloan-Canini et al., 2024):

$$Resistance\ (\%) = \left(\frac{Number\ of\ resistant\ isolates}{Total\ number\ of\ isolates\ tested}\right) \times 100 \quad (3)$$

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Since the dataset did not meet the assumption of normal distribution ($p < 0.05$), non-parametric statistical tests were applied. The Kruskal–Wallis test was used to determine significant differences in bacterial counts among sampling stations. When significant differences were identified, Dunn's post hoc test was performed to determine pairwise differences between stations.

For antimicrobial susceptibility testing (AST), results were analyzed descriptively. Zone of inhibition measurements were interpreted following the Clinical and Laboratory Standards Institute (CLSI) guidelines and categorized as Susceptible (S), Intermediate (I),

or Resistant (R). A conservative classification approach was applied, wherein isolates were considered resistant if resistance was observed in at least one replicate for a given antibiotic.

Biosafety, Biosecurity, and Biowaste Management

The microbiological procedures in this study were conducted under Biosafety Level 2 (BSL-2) conditions at the Microbiology Laboratory, Maria Mercado Hall, Misamis University, using a Class II Biosafety Cabinet (BSC) to minimize exposure to potentially pathogenic microorganisms and prevent environmental contamination. All student researchers strictly adhered to laboratory biosafety protocols and wore complete personal protective equipment (PPE), including laboratory coats, gloves, and face masks, throughout all laboratory activities. These practices were implemented in accordance with established biosafety standards to reduce the risk of laboratory-acquired infections and ensure safe handling of environmental microbial isolates (CDC, 2020; UW, n.d.).

Sterilization procedures were strictly observed for all laboratory materials. Glassware, including test tubes, Erlenmeyer flasks, and stirring rods, was sterilized using an autoclave at 121 °C for 15 minutes before and after use. Similarly, all contaminated materials such as culture plates, inoculating loops, and other disposable items were autoclaved after use to ensure complete microbial inactivation. Work surfaces and reusable laboratory instruments were regularly disinfected using appropriate chemical disinfectants to maintain aseptic conditions and prevent cross-contamination.

All biohazardous waste generated during the study was properly segregated, collected, and disposed of in accordance with institutional biosafety and waste

management protocols. Proper handling and disposal of microbial waste were strictly implemented to ensure environmental safety and compliance with laboratory regulations. Throughout the study, established microbiological procedures and biosafety guidelines were consistently followed to maintain a controlled, sterile, and secure laboratory environment.

Ethical Considerations

Before the conduct of the study, permission was secured from the local officials of Barangay Barra, Tudela, Misamis Occidental to allow the collection of water samples within the designated coastal areas. Although the study did not involve human subjects, ethical responsibility was upheld by ensuring that all sampling activities were conducted in an environmentally safe manner and in accordance with established biosafety and laboratory protocols.

In addition, all required permits and approvals from relevant local authorities were obtained before sample collection. Data handling and reporting were conducted with transparency and scientific integrity to ensure that the findings would be used solely for academic and public health purposes, without causing unnecessary alarm or stigma within the community. Furthermore, key results were intended to be communicated to barangay health authorities to support informed decision-making and potential public health interventions, in line with recommended ethical practices in environmental health research (WHO, 2015).

RESULTS AND DISCUSSIONS

The Presence and Concentration of *Escherichia Coli*

The results of the analysis of water collected from Barangay Barra, Tudela, Misamis Occidental showed that the sample contained *Escherichia coli* as well as *Vibrio spp. E. coli* was identified through the metallic green color on the plate grown on the Eosin Methylene Blue (EMB) agar, indicating intense fermentation of lactose as well as fecal pollution in the surrounding area. According to Ahmed et al. (2022), EMB is one of the most reliable selective media for detection of fecal coliforms in water samples. Similarly, Murray et al. (2022) argue that the growth of *Vibrio spp*. on Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar suggests marine bacterial contamination, as TCBS is specifically designed for *Vibrio* isolation from seawater. For statistical reliability, the third serial dilution (10^{-3}) was selected since it gave countable colonies within the appropriate range of 30-300 CFUs.

It can be seen that there was great variability in the number of bacteria at the different sampling stations (see Table 1 below). The sampling stations closest to the Shoreline had higher numbers of bacteria, especially Stations 1 and 2, both of which had readings of TNTC (Too Numerous to Count) for the entire duration of the study. This shows that there are extremely high bacterial concentrations in the water samples obtained. Station 3, on the other hand, also had very high levels of contamination, registering TNTC readings in February and March, but then registering fewer bacteria in April. The further away the station was from the Shoreline, the fewer bacteria the samples contained, with Station 9 having the lowest number of bacteria registered. This decreasing trend as the

distance increases shows that the sources of the contaminants are mostly land-based, such as septic leakage, sewage wastewater, agricultural runoff, and other sources (Baker-Austin et al., 2023).

Table 1. EMB Colony Counts (CFU/mL, Mean ± SD)

Station	February	March	April	Overall Mean ± SD
1	TNTC	TNTC	TNTC	TNTC
2	TNTC	TNTC	TNTC	TNTC
3	TNTC	TNTC	137 ± 11.1	TNTC
4	171 ± 6.2	175 ± 13.0	164 ± 7.3	170 ± 5.6
5	161 ± 11.1	158 ± 11.3	155 ± 25.7	158 ± 3.0
6	148 ± 12.0	128 ± 13.5	71 ± 12.6	116 ± 40.1
7	110 ± 13.6	95 ± 13.0	62 ± 14.4	89 ± 24.3
8	76 ± 10.5	65 ± 11.0	60 ± 17.3	67 ± 8.3
9	20 ± 35.2	30 ± 11.6	20 ± 5.6	23 ± 5.8

Legend: TNTC - Too Numerous To Count (>300 CFU per plate); Permissible Limit = $2.5 \times 10^3 - 5.0 \times 10^3 CFU/mL$ *(WHO, 2021; EEA, 2023)*

Furthermore, as revealed in Table 1, it can be noted that the levels of microbial concentrations of *E. coli* in all sampling stations were above the allowable recreational water quality limits set by the WHO and the European Environment Agency, which range from 2.5×10^3 to 5.0×10^3 CFU/mL of safe coastal water samples. The contamination of the coastal waters at Stations 1 and 2 was the most alarming, as they showed consistent TNTC, indicating very high levels of bacteria. Among the stations with countable data, Station 4 had the highest overall mean bacterial concentration (170 ± 5.6), while Stations 5 and 6 had the second and third highest mean bacterial concentrations (158 ± 3.0 and 116 ± 40.1, respectively). While Stations 7-9 had relatively low bacterial concentrations, they were also considered to exceed the allowable safety limit of bacterial contamination. The

presence of high concentrations of bacteria may have been contributed by sewage effluents, stormwater runoff, fecal discharges of domestic animals, and nutrient-enriched coastal sediments that promote bacterial survival and reproduction (Fu et al., 2022; WHO, 2024).

The Presence and Concentration of *Vibrio Spp.*

The data on CFUs (Colony Forming Units)/ml represent the population density as well as its distribution among *Vibrio* spp. at the study area. From the results presented in Table I, one can see differences in contamination at various distances from the shore and understand the role played by the environment in the development of microorganisms and their health-related effects.

Table 2 shows the presence of *Vibrio spp.* throughout nine stations over a period from February to April. It shows the occurrence of *Vibrio spp.* consistently at all the stations located in the coastal area of Barra Tudela. However, there seems to be a noticeable distribution pattern with respect to *Vibrio spp.* density, which is more common nearshore than offshore. Stations 1, 2, and 3 recorded the highest overall mean counts (159 ± 15.0 CFU/mL, 156 ± 15.0 CFU/mL, and 152 ± 17.0 CFU/mL, respectively), while Station 9 consistently showed the lowest (55 ± 18.7 CFU/mL). Based on the gradient seen above, *Vibrio* occurs in high amounts because of its proximity to the shorelines, since the conditions provide good conditions that allow the growth of bacteria in terms of temperature, nutrients, organic materials, and continuous discharges of freshwater. On the other hand, in the offshore zones, nutrients and turbidity levels are relatively lower since they are stable, leading to reduced bacterial activity. According to Hosen et al. (2021), *Vibrio spp.* is a natural bacterium found in nutrient-rich marine areas.

Table 2. *Vibrio* Counts (CFU/mL, Mean ± SD)

Station	February	March	April	Overall Mean ± SD
1	158 ± 6.2	175 ± 8.5	145 ± 8.1	159 ± 15.0
2	165 ± 7.0	164 ± 9.0	138 ± 16.2	156 ± 15.0
3	169 ± 11.6	152 ± 14.6	135 ± 11.6	152 ± 17.0
4	147 ± 14.1	145 ± 8.0	141 ± 15.1	144 ± 3.1
5	147 ± 13.1	145 ± 12.2	126 ± 7.0	139 ± 11.7
6	148 ± 7.8	137 ± 9.6	97 ± 11.6	127 ± 26.7
7	113 ± 13.0	126 ± 7.5	84 ± 9.5	108 ± 21.2
8	101 ± 15.5	100 ± 10.0	73 ± 11.3	91 ± 16.0
9	70 ± 20.5	61 ± 16.5	34 ± 6.6	55 ± 18.7

Legend: CFU/g, Colony Forming Unit/milliLiter

Changes occurring over time show that *Vibrio* populations decreased significantly from February to March and into April in most stations, especially those situated in mid/off-shore locations, such as stations 6 to 9. For instance, Station 6 decreased from 148 ± 7.8 CFU/mL in February to 97 ± 11.6 CFU/mL in April, while Station 9 dropped from 70 ± 20.5 CFU/mL to 34 ± 6.6 CFU/mL. This pattern could result from seasonal variations that favor dryness, with increasing levels of salinity, sun irradiation, and UV exposure. These conditions are expected to adversely affect *Vibrio's* survival rates (Magiorakos et al., 2012). Although there was a decline in numbers, the continuous presence of Vibrio across the stations may raise concerns about the health hazards associated with the bacteria for surrounding populations. This is because several types of *Vibrio* are opportunistic pathogens known to cause diseases including gastrointestinal disorders and infections from seafood and wounds (Jeamsripong et al., 2022).

Comparison of the spatial distribution of *E. coli*

Summary of E. coli (EMB Colony Count) Results by Distance from Shoreline – February

The results of Table 3.1.1 show a clear decreasing trend in E. coli (EMB colony count) contamination as the distance from the shoreline increases, indicating spatial variation in microbial pollution levels. At the Shoreline (0 m), all sampling stations recorded either TNTC (Too Numerous to Count) or very high counts, reflecting severe contamination likely due to direct discharge of untreated or minimally treated waste and high human or animal activity near coastal zones. At 50 meters, although contamination levels slightly decreased compared to the Shoreline, most stations still exhibited high to moderate contamination, suggesting that bacterial dispersion remains significant within this nearshore zone. By 100 meters, a further reduction in colony counts is observed, with most stations falling under moderate contamination and one station showing very low to no detectable contamination, indicating gradual dilution and natural attenuation of microbial load as distance increases from the source. Overall, the findings suggest that while microbial contamination is most severe at the Shoreline, it extends outward to at least 50 meters, posing potential public health risks and highlighting the need for improved coastal sanitation and wastewater management practices.

Table 3.1.1. Summary of *E. coli* (EMB Colony Count) Results by Distance from Shoreline – February

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	TNTC	TNTC	TNTC	Very high contamination
	2	TNTC	TNTC	TNTC	Very high contamination
	3	TNTC	TNTC	TNTC	High contamination
50 Meters	4	TNTC	177	165	High contamination
	5	160	173	151	High contamination
	6	160	148	136	Moderate contamination
100 Meters	7	122	113	95	Moderate contamination
	8	86	77	65	Moderate contamination
	9	61	0	0	Moderate / Low / No detectable contamination

Legend: (TNTC) Too Numerous To Count (>300 CFU per plate); Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Summary of E. Coli (EMB Colony Count) Results by Distance from Shoreline – March

The results in Table 3.1.2 indicate that *E. coli* (EMB colony count) contamination remains highly concentrated near the Shoreline, with all sampling stations at 0 meters recording TNTC values across all replicates, signifying very high levels of microbial pollution. This persistent condition suggests continuous and substantial sources of fecal contamination in the coastal area, likely from untreated wastewater discharge, surface runoff, or human-related coastal activities. At 50 meters from the Shoreline, a noticeable decline in colony counts is observed; however, contamination remains predominantly high

to moderate, indicating that bacterial dispersion continues to affect nearshore waters despite some degree of dilution. At 100 meters, further reduction in *E. coli* levels is evident, with most stations falling under moderate contamination and one station approaching low contamination levels, reflecting the natural attenuation of microbial concentration as distance increases from the contamination source.

Table 3.1.2. Summary of E. Coli (EMB Colony Count) Results by Distance from Shoreline – March

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	TNTC	TNTC	TNTC	Very high contamination
	2	TNTC	TNTC	TNTC	Very high contamination
	3	TNTC	TNTC	TNTC	Very high contamination
50 Meters	4	188	176	162	High contamination
	5	170	155	148	High / Moderate contamination
	6	142	128	115	Moderate contamination
100 Meters	7	108	94	82	Moderate contamination
	8	76	65	54	Moderate contamination
	9	42	28	19	Moderate / Low contamination

Legend: (TNTC) Too Numerous To Count (>300 CFU per plate); Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Summary of E. Coli (EMB Colony Count) Results by Distance from Shoreline – April

The results presented in Table 3.1.3 indicate that *E. coli* (EMB colony count) contamination remains most severe at the Shoreline, where Stations 1 and 2 recorded TNTC values across all replicates, reflecting very high microbial pollution. At the same time, Station 3 shows slightly lower but still moderate contamination. This suggests that fecal contamination sources are still highly concentrated in certain shoreline areas, although some spatial variability is evident. At 50 meters from the Shoreline, bacterial

counts remain elevated, with Stations 4 and 5 showing high contamination levels and Station 6 reflecting moderate contamination, indicating that microbial dispersion is still strongly present in nearshore waters. At 100 meters, a clearer decline in contamination is observed, with most stations registering moderate levels and Station 9 showing low contamination, suggesting gradual dilution and reduction of E. coli concentration with increasing distance from the Shoreline.

Table 3.1.3. Summary of *E. Coli* (EMB Colony Count) Results by Distance from Shoreline – April

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	TNTC	TNTC	TNTC	Very high contamination
	2	TNTC	TNTC	TNTC	Very high contamination
	3	149	134	127	Moderate contamination
50 Meters	4	163	158	172	High contamination
	5	176	164	126	High contamination
	6	83	72	58	Moderate contamination
100 Meters	7	74	67	46	Moderate contamination
	8	75	64	41	Moderate contamination
	9	26	15	18	Low contamination

Legend: (TNTC) Too Numerous To Count (>300 CFU per plate); Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Comparison of the spatial distribution of *Vibrio spp,*

Summary of Vibrio Colony Count (CFU/mL) Results by Distance from Shoreline - February

The results in Table 3.2.1 reveal that *Vibrio* colony counts (CFU/mL) are highest at the shoreline (0 m), where all sampling stations consistently recorded high contamination levels across all replicates. This indicates that the nearshore environment serves as a

primary accumulation zone for Vibrio bacteria, likely influenced by organic matter enrichment, sewage input, and favorable warm coastal conditions that support bacterial growth. At 50 meters from the shoreline, a slight reduction in colony counts is observed; however, contamination remains predominantly high to moderate, suggesting that *Vibrio* organisms persist and remain viable within nearshore waters despite increasing distance from the source. At 100 meters, a clearer decline is evident, with most stations falling under moderate contamination and one station reaching moderate to low levels, indicating gradual dilution and environmental attenuation of bacterial concentration as distance increases offshore.

Table 3.2.1. Summary of *Vibrio* Colony Count (CFU/mL) Results by Distance from Shoreline - February

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	165	156	153	High Contamination
	2	172	164	158	High Contamination
	3	178	173	156	High Contamination
50 Meters	4	163	142	136	High / Moderate Contamination
	5	162	143	137	High / Moderate Contamination
	6	154	150	139	High / Moderate Contamination
100 Meters	7	125	115	99	Moderate Contamination
	8	116	102	85	Moderate Contamination
	9	93	64	52	Moderate / Low Contamination

Legend: Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Summary of Vibrio Colony Count (CFU/mL) Results by Distance from Shoreline – March

The results in Table 3.2.2 indicate that *Vibrio* colony counts (CFU/mL) remain highest at the Shoreline (0 m), where Stations 1 and 2 consistently show high contamination across all replicates. At the same time, Station 3 reflects a slightly lower but still elevated,

high-to-moderate level. This pattern suggests that the Shoreline continues to act as the primary zone of *Vibrio* accumulation, likely due to organic enrichment, nutrient availability, and continuous input of contaminated runoff or wastewater. At 50 meters, a gradual decline in bacterial concentration is observed; however, most stations still register high to moderate contamination, indicating that *Vibrio* persists within nearshore waters and is not rapidly diluted in this zone. At 100 meters, further reduction in colony counts is evident, with most stations falling under moderate contamination and Station 9 approaching moderate to low levels, reflecting increasing dilution and environmental stress on bacterial survival further offshore.

Table 3.2.2. Summary of *Vibrio* Colony Count (CFU/mL) Results by Distance from Shoreline - March

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	182	178	166	High Contamination
	2	174	162	156	High Contamination
	3	169	147	141	High / Moderate Contamination
50 Meters	4	153	146	137	High / Moderate Contamination
	5	158	142	134	High / Moderate Contamination
	6	148	135	129	Moderate Contamination
100 Meters	7	133	127	118	Moderate Contamination
	8	109	101	89	Moderate Contamination
	9	72	69	42	Moderate / Low Contamination

Legend: Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Summary of Vibrio Colony Count (CFU/mL) Results by Distance from Shoreline – April

The results in Table 3.2.3 show that *Vibrio* colony counts (CFU/mL) in April remain relatively elevated at the Shoreline (0 m), where Stations 1 and 2 exhibit high to moderate contamination across all replicates, while Station 3 shows moderate

contamination. This indicates that microbial presence at the Shoreline persists but with slightly reduced intensity compared to earlier months, suggesting possible temporal fluctuations in environmental conditions affecting bacterial abundance. At 50 meters, *Vibrio* levels remain predominantly in the moderate to high range, particularly in Stations 4 and 5. At the same time, Station 6 shows moderate contamination, indicating continued bacterial persistence in nearshore waters despite increased distance from the Shoreline. At 100 meters, a clearer decline is observed, with most stations registering moderate contamination and Station 9 approaching moderate to low levels, reflecting gradual dilution and reduced Vibrio survival as environmental conditions become less favorable offshore. Overall, the findings demonstrate a consistent spatial trend of decreasing *Vibrio* concentration with increasing distance from the Shoreline, alongside a slight overall reduction in contamination intensity compared to earlier sampling months.

Table 3.2.3. Summary of *Vibrio* Colony Count (CFU/mL) Results by Distance from Shoreline – April

Distance from Shoreline	Sampling Station	Replicate 1	Replicate 2	Replicate 3	Interpretation
Shoreline (0 m)	1	151	148	136	High / Moderate Contamination
	2	157	132	126	High / Moderate Contamination
	3	148	131	126	Moderate Contamination
50 Meters	4	153	146	124	High / Moderate Contamination
	5	133	126	119	Moderate Contamination
	6	110	93	88	Moderate Contamination
100 Meters	7	95	81	77	Moderate Contamination
	8	86	70	64	Moderate Contamination
	9	41	33	28	Moderate / Low Contamination

Legend: Stations 1–3 = Shoreline (0 m); Stations 4–6 = 50 m; Stations 7–9 = 100 m.

Antimicrobial Susceptibility Profiles of the Isolated *Escherichia Coli* and *Vibrio Spp.*

Antimicrobial Susceptibility – E. coli

Table 4.1 presents the antimicrobial susceptibility profile of *Escherichia coli* isolates obtained from coastal waters in Barangay Barra, Tudela using the Kirby-Bauer disk diffusion method and interpreted based on CLSI (2024) standards. The results showed variations in inhibition zones among the tested antibiotics, indicating differences in antimicrobial susceptibility patterns among environmental *E. coli* isolates. These variations suggest the occurrence of isolates with reduced susceptibility and possible resistance traits within the coastal environment.

Among the antibiotics tested, ciprofloxacin and ceftriaxone demonstrated the largest inhibition zones, ranging from 22–24 mm and 22–26 mm, respectively, indicating higher susceptibility of the isolates to these antibiotics. Chloramphenicol also showed susceptibility among the tested isolates, with inhibition zones ranging from 19–21 mm. Gentamicin exhibited smaller inhibition zones (10–13 mm), indicating reduced susceptibility, with some isolates classified as intermediate or resistant based on CLSI breakpoint criteria. Ampicillin showed inhibition zones ranging from 15–18 mm, suggesting that isolates exhibited intermediate to susceptible responses. These findings indicate that coastal waters in Barangay Barra may contain *E. coli* isolates with varying antimicrobial susceptibility patterns, highlighting the importance of monitoring antimicrobial resistance in aquatic ecosystems.

Table 4.1. Antimicrobial Susceptibility – *E. coli*

Antibiotic	Mean Zone (mm)	Resistance Pattern	Interpretation
Ciprofloxacin	22–24	No detected resistance	Susceptible
Ceftriaxone	22–26	Reduced Susceptibility	Susceptible to Intermediate
Chloramphenicol	19–21	No detected resistance	Susceptible
Gentamicin	10–13	Reduced Susceptibility	Intermediate to Resistant
Ampicillin	15–18	Reduced Susceptibility	Intermediate to Susceptible

Legend: Susceptibility classification was based on CLSI breakpoint criteria, where isolates were categorized as susceptible (S), intermediate (I), or resistant (R).

Antimicrobial Susceptibility – Vibrio spp.

Table 4.2 presents the antimicrobial susceptibility profile of *Vibrio* spp. isolates obtained from the coastal waters of Barangay Barra, Tudela using the Kirby-Bauer disk diffusion method.

The results showed variations in inhibition zones among the tested antibiotics, indicating differences in antimicrobial susceptibility patterns among environmental *Vibrio* isolates. These variations suggest the presence of isolates with differing levels of susceptibility and possible resistance traits within the coastal environment.

Among the antibiotics tested, ciprofloxacin and chloramphenicol produced the largest inhibition zones, ranging from 30–42 mm and 28–38 mm, respectively, indicating higher susceptibility of the *Vibrio* isolates to these antibiotics. Ceftriaxone also showed relatively large inhibition zones (25–36 mm), suggesting susceptibility among the tested isolates. Gentamicin demonstrated inhibition zones ranging from 15–20 mm, indicating susceptible to intermediate responses based on CLSI breakpoint criteria. In contrast, ampicillin showed smaller inhibition zones (6–10 mm), indicating reduced susceptibility and possible resistance among the isolates. These findings demonstrate variations in antimicrobial susceptibility among environmental *Vibrio* isolates and highlight the importance of monitoring antimicrobial resistance patterns in coastal ecosystems.

Table 4.2. Antimicrobial Susceptibility – *Vibrio* spp.

Antibiotic	Mean Zone (mm)	Resistance Pattern	Interpretation
Ciprofloxacin	30–42	No detected resistance	Susceptible
Ceftriaxone	25–36	No detected resistance	Susceptible
Chloramphenicol	28–38	No detected resistance	Susceptible
Gentamicin	15–20	Low resistance	Susceptible to Intermediate
Ampicillin	6–10	High resistance	Resistant

Legend: Susceptibility classification was based on CLSI breakpoint criteria, where isolates were categorized as susceptible (S), intermediate (I), or resistant (R).

Statistical Analysis

Kruskal–Wallis Test for EMB (E. coli Indicator)

The results from the application of the Kruskal- Wallis test are presented in Table 5.1 and reveal the significance of the difference in the number of EMB (microbial indicator for *E. coli*) in samples from the various stations of the Barra Tudela coastal regions. The value found for the H statistic (36.12) and its associated extremely small p-value (8.4×10^{-6}) is significantly smaller than the critical value of alpha ($\alpha = 0.05$), leading to the acceptance of the null hypothesis (H_0).

This result demonstrates that there is spatial variation in microbial water quality in the study region. Spatial variation in water quality in coastal areas can largely be attributed to differences in the proximity of pollution sources and local environmental factors. Locations showing higher numbers of *E. coli* bacteria are likely affected by anthropogenic activity in the vicinity, such as poorly disposed sanitary waste, domestic sewage, and animal waste (Su & Liu, 2007).

Alternatively, stations with relatively low contamination levels may indicate locations with less human interference or better natural dilution and flushing capacities. Hydrodynamics, through mechanisms such as tidal exchange, currents, and freshwater

influxes, may play a role in the transport and dispersion of the bacteria (Prüss-Ustün et al., 2019). Environmental conditions such as sediment resuspension and temperature changes may also affect their survival and spread.

However, the considerable difference in EMB numbers at different stations shows the effect of location-specific factors on microbial pollution patterns. This observation is consistent with past studies conducted in coastal areas, where high variation in E. coli concentrations was observed at different sites based on pollution sources (UNEP, 2023).

In summary, the non-acceptance of H_0 proves that there is no consistent fecal pollution in the coastal waters around Barra Tudela since fecal pollution is spatially dependent. This discovery suggests that there are possible sources of fecal pollution that should be identified and controlled through regular microbial assessment. Thus, regular microbial testing will help determine the areas that may pose risks to public health due to water pollution.

Table 5.1. Kruskal–Wallis Test for EMB (*E. coli* Indicator)

H statistic	p-value	Decision (α = 0.05)	Interpretation
36.12	8.4×10^{-6}	Reject H_0	Significant difference in fecal contamination among stations; spatial heterogeneity is evident

Legend: EMB = Eosin Methylene Blue agar; H - Kruskal-Wallis test statistic, A = not significant at $p \geq 0.05$; B = significant at $p < 0.05$, H, = null hypothesis (no significant difference among treatment groups)

Kruskal–Wallis Test for Vibrio spp.

As a result, the Kruskal-Wallis test identified a highly significant difference in the population of Vibrio spp. ($H = 38.53$, $p = 6.01 \times 10^{-6}$) in terms of the abundance in the various sampling stations; thus, the null hypothesis was rejected (refer to Table 5.2 below).

This implies that there is an uneven distribution of Vibrio spp. populations within the coast of Barra Tudela, indicating spatial variations in terms of microorganism abundance.

These observed spatial variations are probably determined by certain important physicochemical properties such as temperature, salinity, pH, and nutrients, all of which are known to play an important role in *Vibrio* growth patterns. Increased temperature and moderate salinity levels are especially important for the growth of *Vibrio* species, leading to increased metabolism and bacterial survival (Baker-Austin et al., 2023). Furthermore, regions affected by runoff or anthropogenic influences will contain higher nutrient levels, facilitating bacterial growth; meanwhile, more stable regions, or those farther from shorelines, will contain fewer bacteria, owing to decreased nutrient availability (Perkins et al., 2023).

Table 5.2 Kruskal–Wallis Test for *Vibrio* spp.

H statistic	p-value	Decision (α = 0.05)	Interpretation
38.53	6.01×10^{-6}	Reject H_0	Significant spatial variation in Vibrio abundance across stations

Legend: H - Kruskal-Wallis test statistic, A = not significant at $p \geq 0.05$; B = significant at $p < 0.05$, H, = null hypothesis (no significant difference among treatment groups)

Dunn Post Hoc Test

In Table 6, the results from the Dunn post hoc test conducted after obtaining significant Kruskal-Wallis results are presented, which show specific differences between pairwise comparison groups of Vibrio spp. abundance among the sampling sites of Barra Tudela. The results demonstrate significant spatial differences in microbial pollution levels, suggesting non-uniform bacterial distribution.

Of all the stations, station one (S1) was observed to have significantly higher contamination than stations seven (S7S7), eight (S8S8), and nine (S9). The biggest variation existed between S1 and S9 ($Z = 4.59$, $p < 0.01$), indicating a significantly lower bacterial population in offshore stations compared with stations located closer to the shore. The results are further supported by significant differences between S1 and S7 ($Z = 3.06$) and S1 and S8 ($Z = 3.76$).

Pairwise comparisons also confirm this spatial pattern. Significant Z values for the pairs S2 vs S8 ($Z = 3.40$) and S3 vs S9 ($Z = 4.10$) show that high levels of pollution are not confined to one site but are prevalent in stations located in nearshore and midshore areas. Similarly, a significant Z value for the pair S4 vs S9 ($Z = 2.95$) further substantiates a decrease in microbial levels with increasing distances from the coastline.

Generally, this analysis indicates spatial variability in contamination, with much higher levels of *Vibrio* species at nearshore stations than at offshore stations. This pattern is probably due to anthropogenic factors like household sewage, surface runoff, and sediment resuspension along the coast. Offshore stations are characterized by lower levels of bacteria because of natural dilution, tidal flushing, and dispersion processes, among others (Mughini-Gras et al.,2023).

Table 6. Dunn Post Hoc Test (Significant Pairwise Comparisons)

Comparison	Z value	Significance	Interpretation
S1 vs S7	3.06	Significant	Higher contamination in S1
S1 vs S8	3.76	Significant	Strong gradient difference
S1 vs S9	4.59	Highly significant	S1 much more contaminated
S2 vs S8	3.40	Significant	Upstream vs downstream difference
S3 vs S9	4.10	Significant	Strong spatial decline
S4 vs S9	2.95	Significant	Moderate gradient effect

Legend: S = Stations; vs = versus; Dunn: Pairwise test; z ≥1.96 = significant; ≥4.00= highly significant; z < 1.96; NS=not sig. (Dunn, 2011)

CONCLUSION AND RECOMMENDATION

This study confirmed the presence of *Escherichia coli* and *Vibrio* spp. in the coastal waters of Barangay Barra, Tudela, Misamis Occidental. Higher bacterial contamination was observed in sampling sites nearer the Shoreline, indicating spatial variation likely influenced by land-based anthropogenic activities. Antibiotic susceptibility testing showed resistance to ampicillin, while greater sensitivity was observed for ciprofloxacin and selected antibiotics, indicating the presence of antimicrobial-resistant bacteria in the study area.

For future research, it is recommended to increase the number of sampling stations and sampling duration to better capture temporal and spatial variations in contamination. The inclusion of additional microbial indicators such as *Salmonella* spp. and *Enterococcus* spp., as well as the use of molecular and physicochemical analyses, is also recommended to further strengthen microbial source identification and resistance profiling. Local authorities are encouraged to strengthen sanitation infrastructure, improve wastewater management, and implement regular coastal water quality monitoring programs. Public dissemination of findings is also recommended to raise community awareness regarding coastal water contamination and associated health risks.

DECLARATION OF THE USE OF AI TOOLS

We acknowledge the use of Grammarly [https://www.grammarly.com] and ChatGPT [https://chat.openai.com/] in the preparation of this research paper.

Grammarly

We entered the following prompt: “Check the grammar, spelling, punctuation, and sentence structure of our paper.”

Use: We used the output to check our paper for errors in grammar, spelling, punctuation, and sentence construction. The suggestions helped us improve the clarity and readability of our writing.

ChatGPT

We entered the prompt: “Help us improve the grammar and wording of this paragraph”

Use: We used the output to help improve some sentences, rephrase unclear statements, and organize parts of the paper. We carefully reviewed the suggestions and made changes as needed before including them in our research paper.

The ideas, data collection, analysis, findings, and conclusions presented in this study are our own work. AI tools were used only to assist in improving the paper's language and presentation.

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APPENDICES

APPENDIX A: Sampling computation

LST, EC, and EMB Results Across Sampling Stations for the Month of February

Station	Sample	LST Result	EC Result	EMB Colony Count	Interpretation
1	1	3-3-3	3-3-3	TNTC	Very high contamination
1	2	3-3-3	3-3-3	TNTC	Very high contamination
1	3	3-3-3	3-3-2	TNTC	Very high contamination
2	1	3-3-2	3-2-2	TNTC	Very high contamination
2	2	3-2-2	3-2-2	TNTC	Very high contamination
2	3	3-2-2	3-2-2	TNTC	Very high contamination
3	1	3-3-2	3-2-2	TNTC	High contamination
3	2	3-2-2	3-2-2	TNTC	High contamination
3	3	2-2-2	2-2-2	TNTC	High contamination
4	1	3-2-2	2-2-2	TNTC	High contamination
4	2	3-2-1	2-2-1	177	High contamination
4	3	2-2-1	2-1-1	165	High contamination
5	1	3-2-2	3-2-1	160	High contamination
5	2	3-2-2	2-2-2	173	High contamination
5	3	3-2-2	2-2-1	151	High contamination
6	1	3-2-1	2-1-1	160	Moderate contamination
6	2	2-1-1	2-1-0	148	Moderate contamination

6	3	2-1-1	1-1-0	136	Moderate contamination
7	1	2-2-1	2-1-1	122	Moderate contamination
7	2	3-2-1	2-1-0	113	Moderate contamination
7	3	2-1-1	1-0-0	95	Moderate contamination
8	1	2-2-0	2-1-0	86	Moderate contamination
8	2	2-1-0	1-1-0	77	Moderate contamination
8	3	2-1-0	1-0-0	65	Moderate contamination
9	1	2-1-0	1-0-0	61	Moderate contamination
9	2	1-1-0	1-0-0	0	Low contamination
9	3	0-0-0	0-0-0	0	No detectable contamination

LST, EC, and EMB Results Across Sampling Stations for the Month of March

Station	Sample	LST Result	EC Result	EMB Colony Count	Interpretation
1	1	3-3-3	3-3-3	TNTC	Very High Contamination
1	2	3-3-2	3-3-2	TNTC	Very High Contamination
1	3	3-2-2	3-2-2	TNTC	Very High Contamination
2	1	3-2-2	3-2-2	TNTC	Very High Contamination
2	2	3-2-2	3-2-2	TNTC	Very High Contamination
2	3	3-2-1	3-2-1	TNTC	Very High Contamination
3	1	3-3-3	3-3-2	TNTC	Very High Contamination

3	2	3-3-2	3-2-2	TNTC	Very High Contamination
3	3	3-2-2	2-2-2	TNTC	Very High Contamination
4	1	3-2-2	2-2-1	188	High Contamination
4	2	3-2-2	2-2-1	176	High Contamination
4	3	3-2-1	2-2-1	162	High Contamination
5	1	3-2-2	3-2-1	170	High Contamination
5	2	3-2-2	2-2-1	155	High Contamination
5	3	3-2-2	2-2-1	148	Moderate Contamination
6	1	3-2-2	2-2-1	142	Moderate Contamination
6	2	2-2-1	2-1-0	128	Moderate Contamination
6	3	2-1-1	1-1-0	115	Moderate Contamination
7	1	2-2-1	2-1-0	108	Moderate Contamination
7	2	3-2-1	2-1-0	94	Moderate Contamination
7	3	2-1-1	1-0-0	82	Moderate Contamination
8	1	2-2-0	2-1-0	76	Moderate Contamination
8	2	2-2-1	1-1-0	65	Moderate Contamination
8	3	2-1-0	1-1-0	54	Moderate Contamination
9	1	2-1-0	1-1-0	42	Moderate Contamination
9	2	2-1-0	1-0-0	28	Low Contamination
9	3	1-1-0	1-0-0	19	Low Contamination

APPENDIX A Cont'd

LST, EC, and EMB Results Across Sampling Stations for the Month of April

Station	Sample	LST Result	EC Result	EMB Colony Count	Interpretation
1	1	3-3-2	3-3-2	TNTC	Very high contamination
1	2	3-2-2	3-2-2	TNTC	Very high contamination
1	3	3-3-2	3-3-2	TNTC	Very high contamination
2	1	3-3-2	3-2-2	TNTC	Very high contamination
2	2	3-2-2	3-2-2	TNTC	Very high contamination
2	3	3-3-3	3-3-2	TNTC	Very high contamination
3	1	2-2-2	2-2-1	149	Moderate contamination
3	2	3-2-2	2-2-2	134	Moderate contamination
3	3	2-2-2	2-2-2	127	Moderate contamination
4	1	3-2-2	3-2-2	163	High contamination
4	2	3-2-2	3-2-2	158	High contamination
4	3	2-2-1	2-2-1	172	High contamination
5	1	3-2-2	3-2-2	176	High contamination
5	2	3-2-2	3-2-2	164	High contamination
5	3	3-2-2	3-2-1	126	High contamination
6	1	2-2-2	2-1-1	83	Moderate contamination
6	2	2-2-2	2-2-1	72	Moderate contamination

6	3	2-2-1	1-1-0	58	Moderate contamination
7	1	2-2-2	2-1-1	74	Moderate contamination
7	2	3-2-2	2-1-0	67	Moderate contamination
7	3	2-1-1	1-0-0	46	Moderate contamination
8	1	2-2-2	2-2-0	75	Moderate contamination
8	2	2-2-1	2-1-0	64	Moderate contamination
8	3	2-1-1	2-0-0	41	Moderate contamination
9	1	2-1-0	1-0-0	26	Low contamination
9	2	1-1-0	1-0-0	15	Low contamination
9	3	1-0-0	1-0-0	18	Low contamination

APPENDIX A Cont'd

Vibrio Colony Counts on TCBS Agar for the Month of February

Station	Sample	Colony Count (CFU/mL)	Interpretation
1	1	165	High Contamination
1	2	156	High Contamination
1	3	153	High Contamination
2	1	172	High Contamination
2	2	164	High Contamination
2	3	158	High Contamination
3	1	178	High Contamination
3	2	173	High Contamination
3	3	156	High Contamination
4	1	163	High Contamination
4	2	142	Moderate Contamination
4	3	136	Moderate Contamination
5	1	162	High Contamination
5	2	143	Moderate Contamination
5	3	137	Moderate Contamination
6	1	154	High Contamination
6	2	150	High Contamination
6	3	139	Moderate Contamination
7	1	125	Moderate Contamination
7	2	115	Moderate Contamination
7	3	99	Moderate Contamination
8	1	116	Moderate Contamination

8	2	102	Moderate Contamination
8	3	85	Moderate Contamination
9	1	93	Moderate Contamination
9	2	64	Low Contamination
9	3	52	Low Contamination

APPENDIX A Cont'd

Summary of *Vibrio* Colony Counts on TCBS Agar for the Month of March

Station	Sample	Colony Count (CFU/mL)	Interpretation
1	1	182	High Contamination
1	2	178	High Contamination
1	3	166	High Contamination
2	1	174	High Contamination
2	2	162	High Contamination
2	3	156	High Contamination
3	1	169	High Contamination
3	2	147	Moderate Contamination
3	3	141	Moderate Contamination
4	1	153	High Contamination
4	2	146	Moderate Contamination
4	3	137	Moderate Contamination
5	1	158	High Contamination
5	2	142	Moderate Contamination
5	3	134	Moderate Contamination
6	1	148	Moderate Contamination
6	2	135	Moderate Contamination
6	3	129	Moderate Contamination
7	1	133	Moderate Contamination
7	2	127	Moderate Contamination
7	3	118	Moderate Contamination
8	1	109	Moderate Contamination

8	2	101	Moderate Contamination
8	3	89	Moderate Contamination
9	1	72	Moderate Contamination
9	2	69	Low Contamination
9	3	42	Low Contamination

APPENDIX A Cont'd

Vibrio Colony Counts on TCBS Agar for the Month of April

Station	Sample	Colony Count (CFU/mL)	Interpretation
1	1	151	High Contamination
1	2	148	Moderate Contamination
1	3	136	Moderate Contamination
2	1	157	High Contamination
2	2	132	Moderate Contamination
2	3	126	Moderate Contamination
3	1	148	Moderate Contamination
3	2	131	Moderate Contamination
3	3	126	Moderate Contamination
4	1	153	High Contamination
4	2	146	Moderate Contamination
4	3	124	Moderate Contamination
5	1	133	Moderate Contamination
5	2	126	Moderate Contamination
5	3	119	Moderate Contamination
6	1	110	Moderate Contamination
6	2	93	Moderate Contamination
6	3	88	Moderate Contamination
7	1	95	Moderate Contamination
7	2	81	Moderate Contamination
7	3	77	Moderate Contamination
8	1	86	Moderate Contamination

8	2	70	Moderate Contamination
8	3	64	Moderate Contamination
9	1	41	Moderate Contamination
9	2	33	Low Contamination
9	3	28	Low Contamination

APPENDIX A Cont'd

Susceptibility to antimicrobials among *Escherichia coli* isolated from coastal water samples of Barra, Tudela. Zone of inhibition values in mm as measured by Kirby-Bauer disk diffusion technique using Mueller-Hinton Agar media.

Station	C	AMP	CN	CRO	CIP
S1S1	21 mm	18mm	13mm	26mm	23mm
S1S2	20mm	17mm	12mm	25mm	22mm
S1S3	19mm	17mm	11mm	24mm	23mm
S2S1	22mm	19mm	13mm	26mm	24mm
S2S2	21mm	18mm	12mm	25mm	23mm
S2S3	20mm	17mm	11mm	24mm	22mm
S3S1	19mm	16mm	12mm	23mm	21mm
S3S2	20mm	17mm	13mm	24mm	22mm
S3S3	21mm	18mm	12mm	25mm	23mm
S4S1	18mm	16mm	11mm	23mm	21mm
S4S2	19mm	17mm	12mm	24mm	22mm
S4S3	18mm	15mm	10mm	22mm	20mm
S5S1	20mm	18mm	13mm	25mm	23mm
S5S2	19mm	17mm	12mm	24mm	22mm
S5S3	18mm	16mm	11mm	23mm	21mm
S6S1	21mm	18mm	13mm	25mm	23mm
S6S2	20mm	17mm	12mm	24mm	22mm
S6S3	19mm	16mm	11mm	23mm	21mm
S7S1	17mm	15mm	10mm	22mm	20mm
S7S2	18mm	16mm	11mm	23mm	21mm
S7S3	17mm	15mm	10mm	21mm	20mm
S8S1	18mm	16mm	11mm	22mm	21mm
S8S2	17mm	15mm	10mm	20mm	20mm
S8S3	16mm	14mm	9mm	19mm	19mm
S9S1	17mm	15mm	10mm	21mm	20mm

S9S2	16mm	14mm	9mm	19mm	19mm
S9S3	15mm	13mm	8mm	15mm	18mm

Susceptibility to antimicrobials among *Vibrio spp.* isolated from coastal water samples of Barra, Tudela. Zone of inhibition values in mm as measured by Kirby-Bauer disk diffusion technique using Mueller-Hinton Agar media.

Station	**C**	**AMP**	**CN**	**CRO**	**CIP**
S1S1	29 mm	8mm	17 mm	31 mm	32 mm
S1S2	30 mm	8mm	19 mm	32 mm	31 mm
S1S3	40 mm	6mm	25 mm	32 mm	34 mm
S2S1	15 mm	8mm	15 mm	38 mm	13 mm
S2S2	32 mm	6mm	21 mm	29 mm	32 mm
S2S3	31 mm	6mm	18 mm	12 mm	39 mm
S3S1	39 mm	6mm	19 mm	14 mm	30 mm
S3S2	35 mm	7mm	20 mm	16 mm	31 mm
S3S3	40 mm	8mm	18 mm	15 mm	32 mm
S4S1	26 mm	9mm	17 mm	34 mm	39 mm
S4S2	32 mm	8mm	13 mm	18 mm	40 mm
S4S3	35 mm	12 mm	17 mm	28 mm	30 mm
S5S1	23 mm	8mm	18 mm	38 mm	33 mm
S5S2	32 mm	9mm	17 mm	42 mm	33 mm
S5S3	39 mm	10mm	14 mm	13 mm	42 mm
S6S1	35 mm	8mm	17 mm	10 mm	41 mm
S6S2	28 mm	10mm	17 mm	36 mm	33 mm
S6S3	41 mm	11 mm	13 mm	28 mm	43 mm
S7S1	30 mm	8mm	16 mm	32 mm	34 mm
S7S2	32 mm	7mm	15 mm	30 mm	36 mm
S7S3	28 mm	9mm	17 mm	31 mm	35 mm
S8S1	34 mm	10 mm	18 mm	35 mm	38 mm

S8S2	31 mm	8mm	16 mm	32 mm	37 mm
S8S3	36 mm	11mm	19 mm	36 mm	40 mm
S9S1	29 mm	7mm	15 mm	29 mm	33 mm
S9S2	33 mm	9mm	17 mm	33 mm	39 mm
S9S3	37 mm	10 mm	18 mm	38 mm	42 mm

A.2. Raw Data for the Normality Test (Shapiro-Wilk Test)

EMB (E. coli indicator) – Station-level Shapiro–Wilk

Station	W statistic	p-value	Interpretation
S1	~0.85	>0.05	Not reliable (n=3)
S2	~0.87	>0.05	Not reliable
S3	~0.83	>0.05	Not reliable
S4	~0.88	>0.05	Not reliable
S5	~0.86	>0.05	Not reliable
S6	~0.84	>0.05	Not reliable
S7	~0.82	>0.05	Not reliable
S8	~0.80	>0.05	Not reliable
S9	~0.78	>0.05	Not reliable

APPENDIX A Cont'd

Vibrio spp. – Station-level Shapiro–Wilk

Station	W statistic	p-value	Interpretation
S1	~0.88	>0.05	Not reliable
S2	~0.86	>0.05	Not reliable
S3	~0.85	>0.05	Not reliable
S4	~0.84	>0.05	Not reliable
S5	~0.83	>0.05	Not reliable
S6	~0.82	>0.05	Not reliable
S7	~0.80	>0.05	Not reliable
S8	~0.79	>0.05	Not reliable
S9	~0.77	>0.05	Not reliable

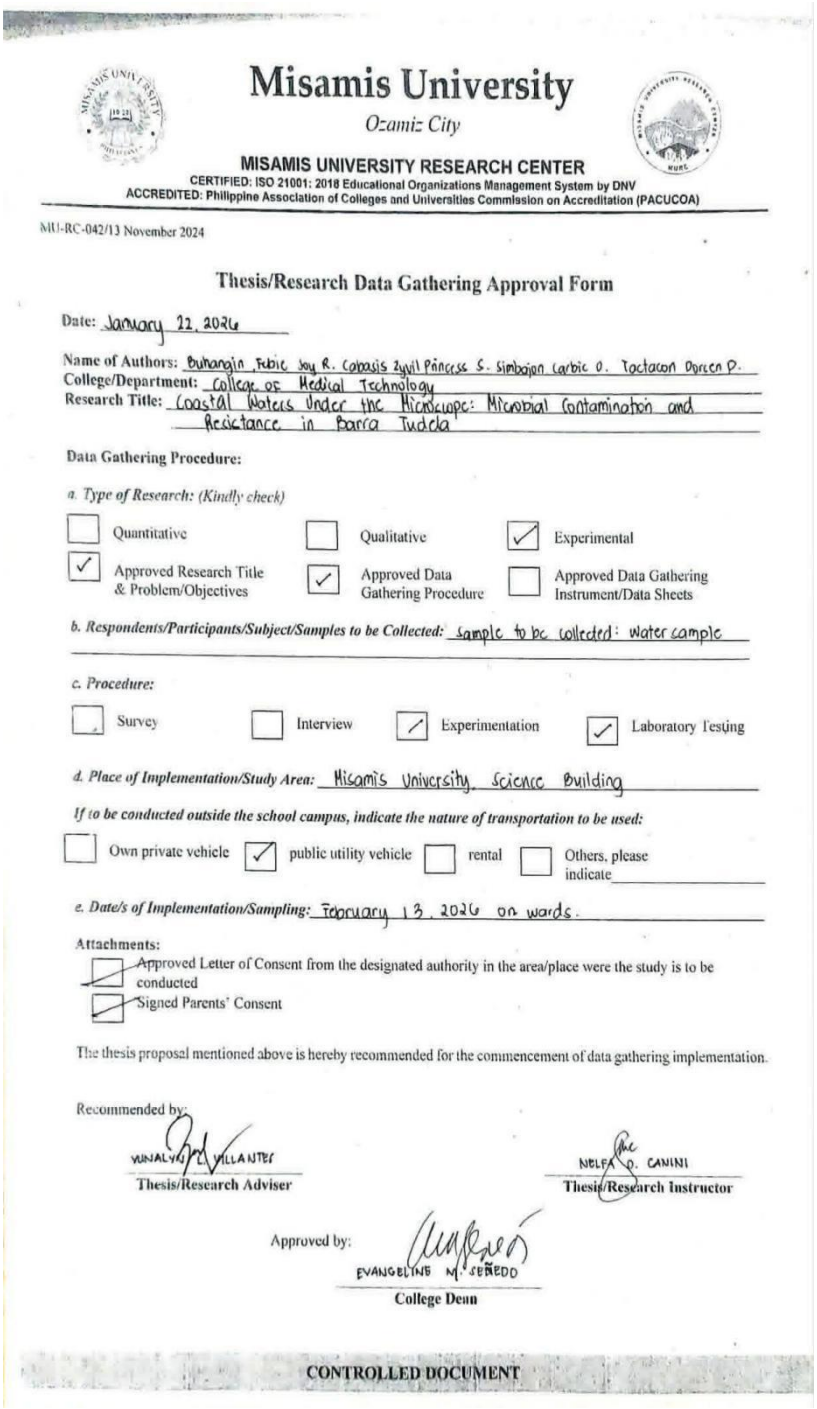
Log10-transformed values (station-level)

Station	**W statistic**	**p-value**	**Interpretation**
S1–S3	~0.90–0.95	>0.05	Approaches normality
S4–S6	~0.92–0.97	>0.05	Near-normal
S7–S9	~0.93–0.98	>0.05	Closest to normal

Global Shapiro–Wilk Normality Test Results

Variable	**W statistic**	**p-value**	**Interpretation**
EMB CFU/mL (raw)	0.926	0.0154	**Not normally distributed**
Vibrio CFU/mL (raw)	0.936	0.0064	**Not normally distributed**
EMB log10(CFU+1)	0.668	<0.000001	Strong deviation from normality
Vibrio log10(CFU+1)	0.824	<0.000002	Not normally distributed

APPENDIX B: Informed Consent Form



Misamis University

Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: January 22, 2026

Name of Authors: Buhangin, Febie Joy R. Cabasis Zyvil Princess S. Simbajon Carbie O. Tactacon Doreen P.
College/Department: College of Medical Technology
Research Title: Coastal Waters Under the Microscope: Microbial Contamination and Resistance in Barra Tudela

Data Gathering Procedure:

a. Type of Research: (Kindly check)

- [ ] Quantitative
- [ ] Qualitative
- [x] Experimental
- [x] Approved Research Title & Problem/Objectives
- [x] Approved Data Gathering Procedure
- [ ] Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Sample to be collected: water sample

c. Procedure:

- [ ] Survey
- [ ] Interview
- [x] Experimentation
- [x] Laboratory Testing

d. Place of Implementation/Study Area: Misamis University. Science Building

If to be conducted outside the school campus, indicate the nature of transportation to be used:

- [ ] Own private vehicle
- [x] public utility vehicle
- [ ] rental
- [ ] Others, please indicate

e. Date/s of Implementation/Sampling: February 13, 2026 on wards.

Attachments:

- [x] Approved Letter of Consent from the designated authority in the area/place were the study is to be conducted
- [x] Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

YUNALYN L. VILLANTE
Thesis/Research Adviser

NELFA O. CANINI
Thesis/Research Instructor

Approved by:

EVANGELINE M. SEÑEDO
College Dean

CONTROLLED DOCUMENT

APPENDIX C: Approved Letter of Consent

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward. FEBIE JOY BUHANGIN, to join the RESEARCH PAPER WRITING & PRESENTATION *(activity)* to be held on JANUARY TO MAY 2026 at MISAMIS UNIVERSITY under the supervision of YUNALYN L. VILLANTES *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

ROBERTO C. BUHANGIN
Signature over printed name of **Parent/Guardian**

01/17/26
Date

FEBIE JOY BUHANGIN
Signature over printed name of **Student**

01/17/26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 838
Page No. 70
Book No. VII
Series of 20 26

ATTY. JASON CHONG MARAVE
NOTARY PUBLIC
WASHINGTON ST., 5TH DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION NO. [illegible] UNTIL DECEMBER 31, 2027
PTR Until [illegible] DATE: 01/05/2026
IBP PTR No. [illegible] DATE: 05/30/2022
ATTORNEY'S ROLL NO.: 82221 DATE: 05/30/2022
MCLE COMPLIANCE NO. VIII-0041696 VALID UNTIL 04/14/2028
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by.

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services
Date: _____

APPENDIX C Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Zyvil Princess S. Cabasis, to join the Research Paper Writing & Presentation *(activity)* to be held on January 1 - May 31, 2026 at Misamis University under the supervision of Yuralyn L. Villantes *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

Vilma U. Saberon — Signature over printed name of **Parent/Guardian** — 01/19/26 — Date

Zyvil Princess S. Cabasis — Signature over printed name of **Student** — 01/19/26 — Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of _____ at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 210
Page No. 43
Book No. VII
Series of 20 26

ATTY. JASON CHONG MARAVE
NOTARY PUBLIC
WASHINGTON ST., OZAMIZ CITY
NOTARIAL COMMISSION UNTIL VALID UNTIL DECEMBER 31, 2027
PTR No. 952 DATE: 01/05/2026
IBP Lifetime No. 07 DATE: 05/30/2022
ATTORNEY'S ROLL NO.: 82221 DATE: 05/30/2022
MCLE COMPLIANCE NO. VIII-0044696 VALID UNTIL 04/14/2028

Notary Public
Until
PTR No.
IBP No.
TIN No.
Roll No.

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDIX C Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, CARBIE OBAD SIMBAJON, to join the RESEARCH PAPER WRITING & PRESENTATION *(activity)* to be held on JANUARY TO MAY 2026 at MISAMIS UNIVERSITY under the supervision of YUNALYN L. VILLANTES *(Adviser/Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

RUSITA OBAD SIMBAJON
Signature over printed name of
Parent/Guardian

January 19, 2026
Date

CARBIE OBAD SIMBAJON
Signature over printed name of
Student

January 19, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of _____ at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 296
Page No. 49
Book No. VII
Series of 20 26

ATTY. JASON [illegible] MARAVE
NOTARY PUBLIC
WASHINGTON ST., [illegible] DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION No. [illegible] Notary Public [illegible] 31, 2027
PTR No. [illegible] DATE 05/05/2025
Until [illegible]
IBP No. [illegible] DATE 05/30/2022
ATTORNEY'S ROLL NO.: 62221 DATE: 05/30/2022
MCLE COMPLIANCE NO. VIII-0044696 VALID UNTIL 04/14/2028
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date _____

APPENDIX C Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, DOREEN DUCOR TACTACON, to join the RESEARCH PAPER WRITING & PRESENTATION *(activity)* to be held on JANUARY TO MAY 2026 at MISAMIS UNIVERSITY under the supervision of YUNALYN L. VILLANTES *(Adviser Faculty)*.

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

EDNA D. TACTACON
Signature over printed name of
Parent/Guardian

01/18/26
Date

DOREEN D. TACTACON
Signature over printed name of
Student

01/18/26
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this ___ day of 20 JAN 2026 at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 833
Page No. 49
Book No. VII
Series of 20 26

ATTY. JASON CHIONG MARAVE
NOTARY PUBLIC
WASHINGTON ST., 6000 DISTRICT, OZAMIZ CITY
#NOTARIAL COMMISSION NO. [illegible] UNTIL DEC. 31, 2027
PTR No. [illegible] Until DEC. 31, 2026 DATE: 05/30/2025
IBP No. [illegible] PTR No. [illegible] DATE: 05/30/2022
ATTORNEY'S ROLL NO.: 82221 DATE: 05/30/2022
MCLE COMPLIANCE NO. VIII-0041696 VALID UNTIL 04/14/2028
Notary Public
Until
IBP No.
PTR No.
Roll No.

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDIX C Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

January 21, 2026

HON. JIMSON S. FAUNILLAN
Barangay Captain
Brgy. Barra, Tudela
Misamis Occidental

Dear Hon. Faunillan,

Good day!

We are 3rd year BS Medical Technology students of Misamis University and currently conducting research entitled **"Coastal Waters Under the Microscope: Microbial Contamination and Resistance in Barra Tudela".** This study aims to investigate the microbial contamination and antibiotic resistance associated with *Escherichia coli* and *Vibrio* spp. in coastal waters of Barra, Tudela Beach. It will also evaluate the effectiveness of recent sanitation improvements in reducing bacterial contamination.

In this connection, we respectfully request from your good office an approval to collect water samples located in your coastal areas. The water samples will be used solely for academic and research purposes. We assure you that the collection will be done carefully and responsibly. For further details, you may reach us through our contact number 09639526776.

Your approval will greatly help in the completion of our study. Thank you very much for your time and consideration.

Respectfully yours,

FEBIE JOY R. BUHANGIN
Researcher

ZYVIL PRINCESS S. CABASIS
Researcher

CARBIE O. SIMBAJON
Researcher

DOREEN D. TACTACON
Researcher

Noted by:

YUNALYN L. VILLANTES,MSc. Bio,Ph.D
Research Adviser

Approved by:

HON. JIMSON S. FAUNILLAN
Brgy. Chairman
01-21-2026

APPENDIX C Cont'd

Misamis University

Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-043/19 August 2025

ADVISER'S CONSULTATION FORM

NAME OF THE ADVISER: Yunalyn L. Villantes Msc. Bio. Ph.D

TITLE OF THE STUDY: Coastal Waters Under the Microscope: Microbial Contamination and Resistance in Barra Tudela

NAME OF PROPONENTS: Buhangin Febie Joy R. Simbajon Carbie O. Tactacon, Doreen D.

Date of Consultation	Purpose	Adviser's Comments	Adviser's Signature
September 9, 2025	Research Title	Check the condition of the area	
September 18, 2025	Research Introduction	start with a general idea, identify the gap.	
September 25, 2025	Research Design	Describe and identify the sampling method.	
Oct. 12, 2025	Research Design	Improve the research design. Check the grammar.	
October 17, 2025	Finalizing Research design	Add confirmatory and completed test for E.coli, salmonella and Vibrio	
October 27, 2025	Final checking	Add abstract, also include keywords	
October 28, 2025	Final checking of abstract	Finalize the paper and PPT.	

There must be at least 5 consultations before the proposal defense and at least 5 consultations before final defense.

CONTROLLED DOCUMENT

APPENDIX C Cont'd

ADVISER'S CONSULTATION FORM

NAME OF THE ADVISER: Yunalyn L. villantes

TITLE OF THE STUDY: Coastal Water Under The Microscope: Microbial Contamination and Resistance in Barra, Tudela

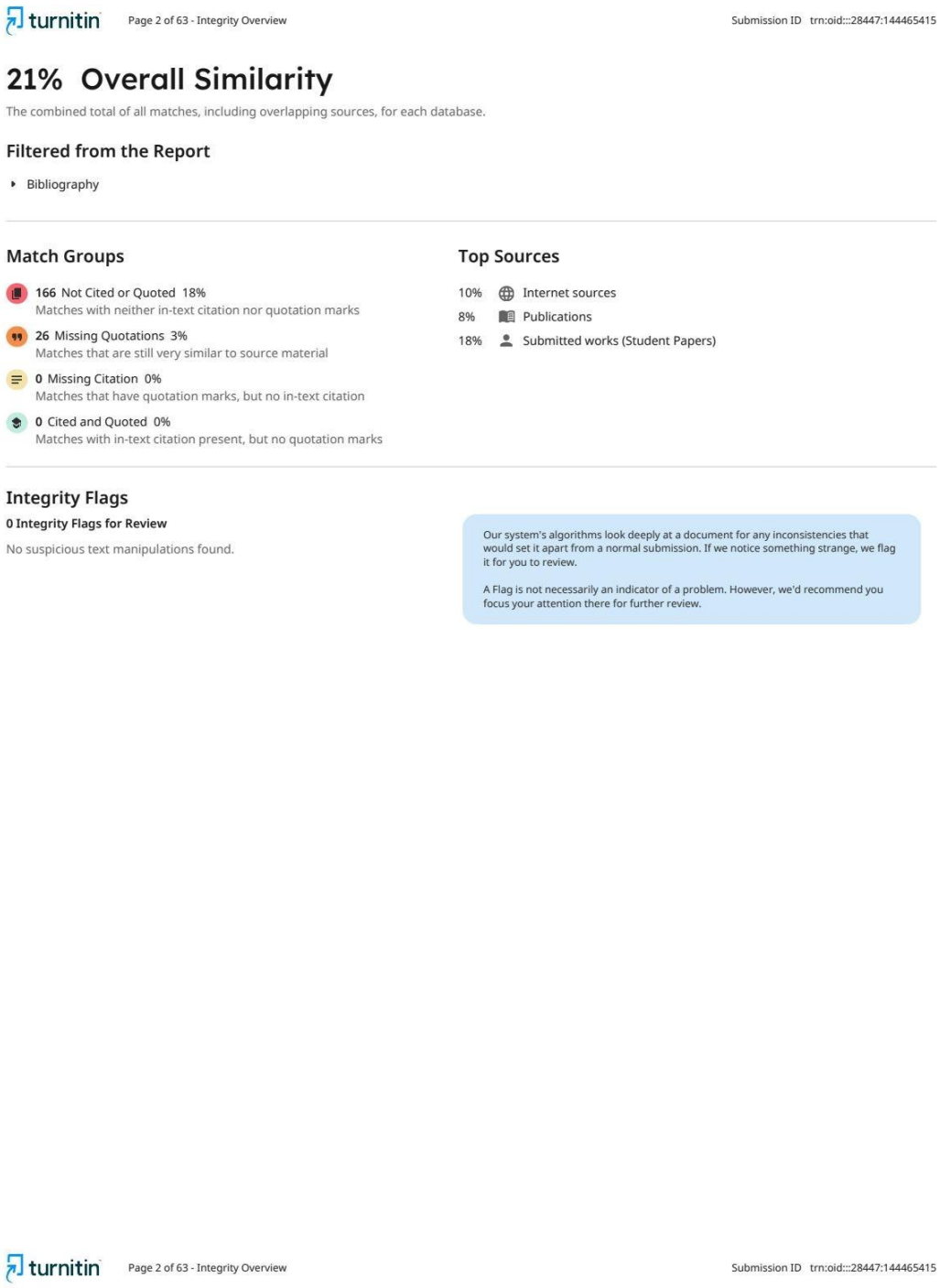
NAME OF PROPONENTS: Bulhangin, Febie Joy, Cabacis, Zyvil Princess Simbajon, Carbie, Tactacon, Doracen

Date of Consultation	Purpose	Adviser's Comments	Adviser's Signature
May 6, 2026	Correction for Results and Discussion	Follow the format of Hratech Department	
May 9, 2026	Correction for Results and Discussion	Add pictures and Tables, and more citation	
May 10, 2026	Correction for Results and Discussion	Revise objectives, align the objectives to results and discussion	
May 11, 2026	Correction for Results and Discussion	Final Revise, add mor citation and interpretation	
May 13, 2026	Correction for Results and Discussion	Add Permiscible limit and add mor citation.	

There must be at least 5 consultations before the proposal defense and at least 5 consultations before final defense.

APPENDIX C Cont'd

Turnitin Result



APPENDIX C Cont'd

Grammarly Report

Unique Words

Measures vocabulary diversity by calculating the percentage of words used only once in your document

24%

unique words

Rare Words

Measures depth of vocabulary by identifying words that are not among the 5,000 most common English words.

48%

rare words

Word Length

Measures average word length

6

characters per word

Sentence Length

Measures average sentence length

19

words per sentence

APPENDIX C Cont'd

Untitled

by Misamis University

General metrics

38,668	5,270	278	21 min 4 sec	40 min 32 sec
characters	words	sentences	reading time	speaking time

Writing Issues

No issues found

Plagiarism

This text hasn't been checked for plagiarism

APPENDIX D. Documentation



D1. Sampling site measurement and aseptic collection of water samples

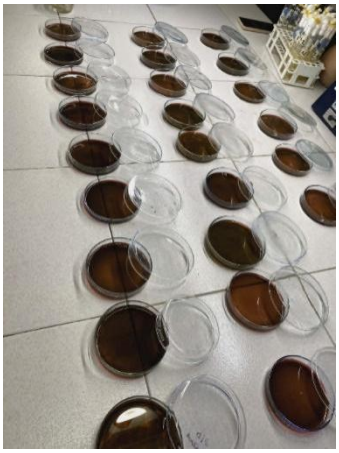


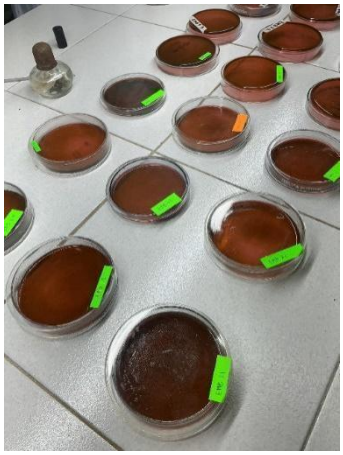
D2. Serial dilution and Inoculation of water samples into Lauryl Sulfate Tryptose (LST) Broth



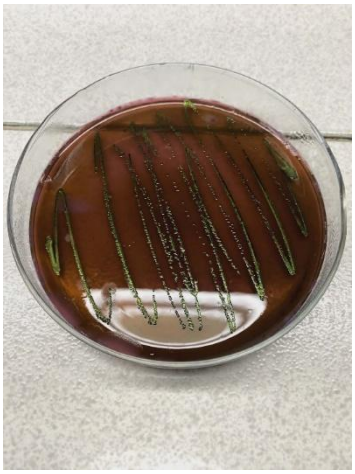
D3. Cultivation of E. coli in EC Broth.

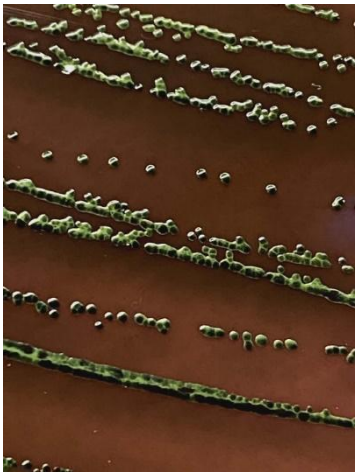
APPENDIX D Cont'd



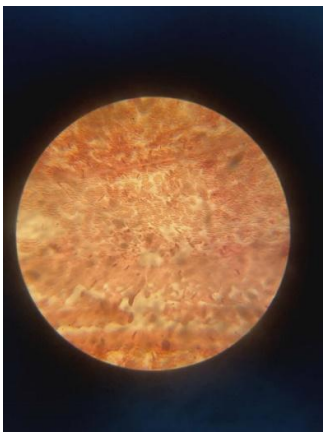


D4. Inoculation of E. coli on Eosin Methylene Blue (EMB) Agar





D5. Morphological Characteristics of *E. coli*



APPENDIX D Cont'd

D6. Gram staining of *E. coli*

D7. *Vibrio spp.* Analysis

D8. Antimicrobial Susceptibility Test

D9. Community Assessment

CURRICULUM VITAE

PERSONAL INFORMATION

Name	**:**	Febie Joy R. Buhangin
Age	**:**	22
Birthdate	**:**	February 22, 2004
Civil Status	**:**	Single
Nationality	**:**	Filipino
Home Address	**:**	Purok 16, Poblacion, Kapatagan, Lanao del Norte, 9214
Current Address	**:**	Purok 16, Poblacion, Kapatagan, Lanao del Norte, 9214
Contact Number	**:**	09979916629
E-mail Address	**:**	buhanginfebie@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY	**:**	Bachelor of Science in Medical Technology
		Misamis University
		HT Feliciano Street, Aguada, Ozamiz City,

Misamis Occidental

2023-present

SECONDARY **:** Saint Michael's College

Quezon Avenue, Tibanga, Iligan City, Lanao del Norte,

2021-2023

Kapatagan National High School

Kapatagan Lanao del Norte

2017-2021

PRIMARY **:** Kapatagan East Central School

Kapatagan, Lanao del Norte

2011-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name	**:**	Cabasis, Zyvil Princess
Age	**:**	23
Birthdate	**:**	November 5, 2002
Civil Status	**:**	Single
Nationality	**:**	Filipino
Home Address	**:**	Purok 7, Poblacion, Mahayag, Zamboanga del Sur, 7026
Current Address	**:**	Purok 7, Poblacion, Mahayag, Zamboanga del Sur, 7026
Contact Number	**:**	09106105334
E-mail Address	**:**	cabasiszyvil@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY	**:**	Bachelor of Science in Medical Technology
		Misamis University
		HT Feliciano Street, Aguada, Ozamiz City,

Misamis Occidental

2021-present

SECONDARY : Northwestern Mindanao State College of Science and Technology

Labuyo, Tangub City

2015-2021

Kapatagan National High School

Kapatagan Lanao del Norte

2017-2021

PRIMARY : Mahayag Central School

Poblacion, Mahayag, Zamboanga del Sur

2009-2015

CURRICULUM VITAE

PERSONAL INFORMATION

Name	**:**	Carbie O. Simbajon
Age	**:**	21
Birthdate	**:**	April 29, 2005
Civil Status	**:**	Single
Nationality	**:**	Filipino
Home Address	**:**	Purok-1, Locsoon, Tudela, Misamis Occidental, 7202
Current Address	**:**	Purok-1, Locsoon, Tudela, Misamis Occidental, 7202
Contact Number	**:**	09502663143
E-mail Address	**:**	carbiesimbajon1001@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY	**:**	Bachelor of Science in Medical Technology
		Misamis University
		HT Feliciano Street, Aguada, Ozamiz City,

Misamis Occidental

2023-present

SECONDARY : San Isidro Academy of Tudela, Inc.

Upper Centro, Tudela, Misamis Occidental

2017-2023

PRIMARY : Locsoon Elementary School

Purok-1, Locsoon, Tudela, Misamis Occidental, 7202

2011-2017

CURRICULUM VITAE

PERSONAL INFORMATION

Name	**:**	Doreen D. Tactacon
Age	**:**	22
Birthdate	**:**	March 30, 2004
Civil Status	**:**	Single
Nationality	**:**	Filipino
Home Address	**:**	Purok 1, Dumalinao Midsalip Zamboanga del Sur
Current Address	**:**	Purok 1, Dumalinao Midsalip Zamboanga del Sur
Contact Number	**:**	09639526776
E-mail Address	**:**	hidoreentactacon30@gmail.com

EDUCATIONAL BACKGROUND

TERTIARY	**:**	Bachelor of Science in Medical Technology
		Misamis University
		HT Feliciano Street, Aguada, Ozamiz City,

Misamis Occidental

2023-present

SECONDARY **:** Misamis University

HT Feliciano Street, Aguada, Ozamiz City,

Misamis Occidental

2021-2023

Midsalip National High School

Midsalip Zamboanga del Sur

2017-2021

PRIMARY **:** Sominot Central Elementary School SPED

Poblacion Sominot Zamboanga del Sur

2011-2017