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CONTAMINATION OF *Escherichia coli* AND *Salmonella* spp. IN RABBITFISH (*Siganus guttatus*) COLLECTED IN MALAUBANG, OZAMIZ CITY

Researchers:

BARONG, RAVEN SHEIN M.

ENGRACIA, TRISIA NICHOLE L.

GODINEZ, TASHA MAE CHARISSE N.

Adviser:

JONAS T. HINGCO, MSc.Bio

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CERTIFICATE OF PANEL APPROVAL

The research entitled **"CONTAMINATION OF *Escherichia coli* AND *Salmonella* spp. IN RABBITFISH (*Siganus guttatus*) COLLECTED IN MALAUBANG, OZAMIZ CITY"** prepared and submitted by **Raven Shein M. Barong, Trisia Nichole L. Engracia,** and **Tasha Mae Charisse N. Godinez**. In partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, has been examined and recommended for acceptance and approval for **ORAL EXAMINATION**.

Approved by the Committee on Oral Examination with a grade of _____

JONAS T. HINGCO, MSc.Bio
Research Adviser

5/22/25
Date

KARL MAXELL O. LAO, RMT, MSc. MLS
Panel Member

5/22/25
Date

YUNALYN L. VILLANTES, MSc. Bio, Ph.D
Panel Member

5/22/2025
Date

ATTY. ANTHONY L. AWA, MSc. Bio, LPT
Panel Member

5/22/25
Date

EVANGELINE M. SEÑEDO, RMT, MATMRS
College Dean

5-15-25
Date

ABSTRACT

Microbial contamination in seafood posed a critical public health concern due to the potential presence of enteric pathogens. This study aimed to determine the presence and load of *Escherichia coli* and *Salmonella* spp. in rabbitfish and to assess their antibiotic resistance profiles. A descriptive experimental design was used. Rabbitfish samples were collected through convenience sampling during the wet season. Microbiological testing included culture on EMB and SSA, biochemical confirmation, and Gram staining. Antibiotic susceptibility testing was conducted via the Kirby-Bauer disk diffusion method using five antibiotic classes. Results showed that all five samples were positive for *Escherichia coli* and *Salmonella* spp. with counts exceeding 10^8 to 10^{10} CFU/g. Sample 2 had the highest *Escherichia coli* load (3.61×10^{10} CFU/g), and Sample 3 had the highest *Salmonella* spp. count (2.84×10^{10} CFU/g). All isolates showed complete resistance to ampicillin, with variable resistance patterns observed for gentamicin and chloramphenicol. Ciprofloxacin remained the most effective antibiotic against both pathogens. These findings suggest notable fecal contamination and the presence of multidrug-resistant bacteria in rabbitfish from Malaubang. The study emphasized the urgent need for improved sanitation, antibiotic use regulation, and routine seafood monitoring to reduce foodborne illness risks.

Keywords: antimicrobial resistance, bacterial load, infectious risk, foodborne, sanitation practices

DEDICATION

This research study is wholeheartedly dedicated all those who have been instrumental in our academic journey and to the people whose lives we hope to positively impact through this work.

To our families, who served as our foundation throughout this research— thank you for your unwavering support, patience, and love. Your constant encouragement inspired us to persevere even during the most challenging times.

To our instructors and research adviser, your guidance, knowledge, and commitment to our growth made this study possible. Your belief in our capabilities gave us the courage to push boundaries and embrace new learning experiences.

To the local fishermen and community of Malaubang, Ozamiz City, this research is dedicated to you. Your cooperation and contribution allowed us to pursue this study, and we hope that our findings will bring light to the importance of food safety and environmental protection within your community.

To our fellow students and research partners, thank you for the teamwork, shared ideas, and the many hours of effort poured into this endeavor.

Lastly, to our Almighty God, thank you for the wisdom, strength, and guidance. You have given us throughout this research journey. This accomplishment is a reflection of Your grace. This study is a symbol of our collective perseverance and a humble contribution to the fields of science and public health.

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INTRODUCTION

Background of the Study

Marine fish are a significant human food source in diets because they are very rich in nutrients. They have plenty of iron, zinc, iodine, magnesium, potassium, protein, calcium, phosphorus, omega-3 fatty acids, and vitamins D and B_2 (riboflavin). This is because they provide food security, employment opportunities, and other benefits, marine fisheries are vital to the economy and well-being of coastal communities. The economy and general well-being of coastal towns are greatly influenced by marine fisheries, which offer jobs, food security, livelihoods, earnings, and preserving one's traditional cultural identity. High-quality protein can be obtained at a reasonable price from fish and fisheries products. Between the 1960s and 2020, the amount of fish consumed per person worldwide climbed from 9.9 kg to 20.2 kg (Zacharia & Issa, 2023).

The most significant source of high-quality protein and an essential food source for humans is fish. Around 16% of the animal protein consumed by people worldwide comes from fish. According to reports, the fishing sites and surrounding environmental elements have an impact on the microbial richness of fresh fish muscle (Mhongole & Mdegela, 2022). Many fish that are commonly consumed are susceptible to pathogenic spoilage, particularly by certain microorganisms. To safeguard the public's health, it is critical to determine the microbiological quality of the fish we usually eat (Faridullah et al., 2022).

Infections associated with food are still a major global public health concern, especially in areas with poor waste management and environmental monitoring. Because

of the exposure to contaminated waters, seafood is commonly linked to microbial contamination. *Escherichia coli* and *Salmonella* spp. are two of the most common pathogens that can be seen in seafood and are both known to cause serious gastrointestinal infections (Roongrojmongkhon et al., 2022). Aquatic species like rabbitfish (*Siganus spp.*)—a marine fish found throughout the Indo-Pacific region, particularly in coral-rich coastal areas, mangroves, and seagrass beds—are contaminated by these bacteria, which are frequently introduced into marine ecosystems by human and animal waste, particularly in places without adequate waste management systems (Oleastro et al., 2022).

In coastal areas like Malaubang, Ozamiz City, Philippines, where rabbitfish are frequently collected and eaten, the risk of contamination is rising. This is particularly dangerous in areas where untreated wastewater could seep into the marine environment and foster the development of harmful microorganisms. Because of these, eating raw or undercooked seafood, such rabbitfish, increases the risk of contracting foodborne illnesses (Boutaib et al., 2020). The threats to one's health that *Escherichia coli* and *Salmonella* spp. are made worse by their increasing antibiotic resistance, which makes it more difficult for affected people to receive treatment (Roongrojmongkhon et al., 2022). An additional cause for concern for public health is the growth in multidrug-resistant strains of these diseases in seafood, according to studies (Oleastro et al., 2022).

Antibiotics have been used extensively as an animal growth enhancer, regardless of whether a bacterial infection has been identified, and are frequently given to treat infections in both humans and animals (Jian et al., 2021). Until the invention of antibiotics, bacterial diseases like meningitis and bacteremia were incurable and hence deadly. Now, however, they can be treated. Antibiotic-resistant bacteria have unfortunately spread more

quickly in recent decades due to social and economic causes, abuse, and misuse of antibiotics, rendering medical treatment useless (Mancuso et al., 2021). *Escherichia coli* and *Salmonella* spp. are two of the most common zoonotic pathogens found in food. The growing prevalence of antibiotic resistance in these pathogens are a major global public health concern (Mumbo et al., 2023).

With the Food and Agricultural Organization (FAO) projecting that worldwide seafood production would reach 179 million tons in 2020, with aquaculture making up over half of this total, the significance of seafood in global nutrition cannot be overstated. (FAO, 2022). Food security is greatly enhanced by seafood's provision of vital elements such vitamins, minerals, and omega-3 fatty acids, especially in coastal populations (FAO, 2022). On the other hand, the dangers of eating tainted seafood emphasize the necessity of efficient environmental surveillance and food safety protocols. Because rabbitfish is a common and reasonably priced food source in Malaubang, Ozamiz City it is important to thoroughly inspect it for any potential microbiological dangers, especially considering the region's weak waste management infrastructure and susceptibilities to environmental threats. However, while *Escherichia coli* and *Salmonella* spp. had been studied in various aquatic species, limited research focused on rabbitfish in specific local environments, such as Malaubang, Ozamiz City. The prevalence of these pathogens in local fish species and their resistance profiles in this area remained largely undocumented. The ecological consequences of disease spread in regional aquaculture systems were also poorly understood.

Objectives

This study aimed to investigate the microbiological contamination and antibiotic resistance profiles associated with rabbitfish (*Siganus guttatus*) in Malaubang, Ozamiz City, to assess potential public health risks and environmental factors contributing to contamination. Specifically, the study aimed to:

1. Detect and quantify the bacterial contamination of *Escherichia coli* and *Salmonella* spp. in rabbitfish (*Siganus guttatus*) collected from Malaubang, Ozamiz City by determining their presence and colony-forming units (CFUs);
2. Confirm the identity of *Escherichia coli* and *Salmonella* spp. isolates through biochemical tests, including Gram staining, Lysine Iron Agar (LIA), and Triple Sugar Iron (TSI) tests; and
3. Assess the antibiotic resistance profiles of *Escherichia coli* and *Salmonella* spp. isolates from rabbitfish samples using Antimicrobial Susceptibility Testing (AST) by measuring the zones of inhibition (ZOI) for various antibiotics.

Significance of the Study

The results of this study revealed significant knowledge about the existence of microbial contaminants in rabbitfish. Local public health officials in Malaubang, Ozamiz City could use the study's findings to develop more stringent environmental regulations and food safety standards, particularly in relation to the discharge of waste into coastal seas. Understanding the ecological consequences of microbial contamination in aquaculture was crucial to safeguarding consumers against foodborne illnesses. Additionally, Antimicrobial Susceptibility Testing (AST) was included to provide crucial

information on the resistance patterns of *Escherichia coli* and *Salmonella* spp., providing insights into the possible treatment difficulties these pathogens may present. In order to address the larger problem of antimicrobial resistance, the AST results aided in the development of well-informed decisions about the use of antibiotics in clinical settings as well as practices connected to fisheries. This study contributed to the scant literature on microbial contamination in rabbitfish and emphasized the dangers to public health posed by improper waste management in coastal areas.

Scope and Delimitation

This investigation was carried out to ascertain microbiological contamination of rabbitfish (*Siganus guttatus*) in Malaubang, Ozamiz City, specifically targeting the presence of *Escherichia coli* and *Salmonella* spp., along with their antibiotic resistance profiles. It underlined the importance of monitoring these microbial infections in aquatic habitats for both ecological and public health reasons, focusing on the local implications for ecosystem health and food safety. By supplying information on the efficacy of widely used antibiotics against the isolated bacteria, Antimicrobial Susceptibility Testing (AST) broadened the study's focus and provided insight into the dangers associated with antimicrobial resistance (AMR).

MATERIALS AND METHODS

Research Design

This research used a descriptive experimental research design that integrated laboratory-based experimentation and quantitative analysis. The study sought to identify, enumerate, and examine the occurrence and antimicrobial resistance patterns of *Escherichia coli* and *Salmonella* spp. in rabbitfish (*Siganus guttatus*) obtained from Malaubang, Ozamiz City.

Research Setting

The study was conducted in the Microbiology Laboratory Maria Mercado Hall and Natural Science Laboratory at Misamis University. The equipment and sterile environment needed to process the samples and carry out the microbiological tests required to identify *Escherichia coli* and *Salmonella* spp. were available at this site.

Study Area

The samples were collected in Malaubang, Ozamiz City, specifically in Boulevard New Road, a coastal community identified as an endemic area for rabbitfish (*Siganus guttatus*) has been documented in the Panguil Bay area, which includes Malaubang, Ozamiz City, indicating its presence in this region (Gonzaga, 2020). It was selected due to its proximity—about 10 minutes from the city proper—and importance to the local fishing industry. Residents utilized the surrounding coastal waters for subsistence fishing,

contributing to the local economy. Coastal households near fishing grounds reflected the connection between the local community and marine resources.

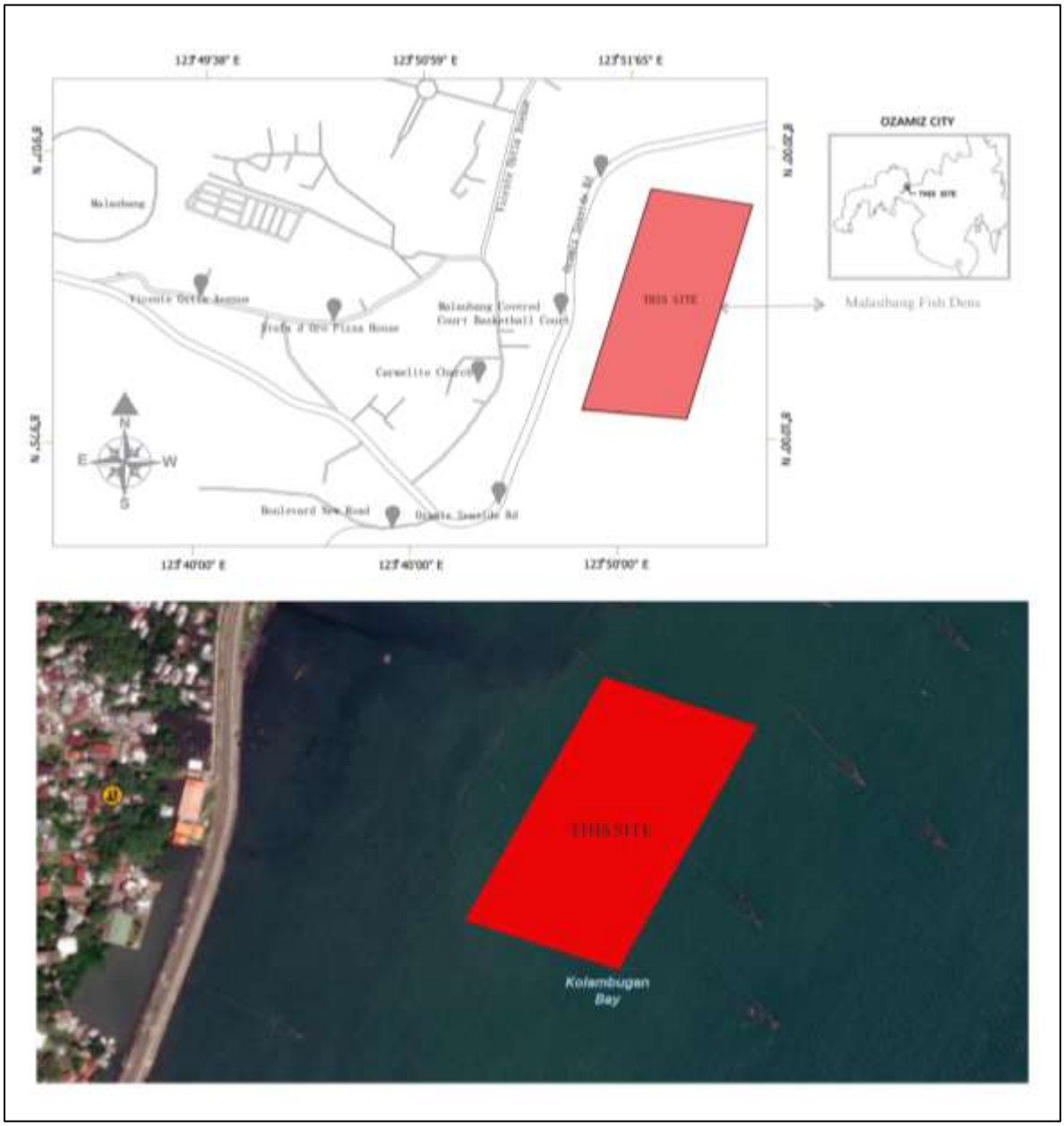

Figure 1. The sampling site of the study in Boulevard, Malaubang, Ozamiz City

While the entire site was considered, sample collection was concentrated in areas that were more accessible to the researchers. These areas were often near landing zones, residential vicinities, and commonly used fishing spots.

As shown in Figure B, three fish dens served as collection points with the following coordinates: the first fish den (i) at 8.130806, 123.834423; the second fish den (ii) at 8.131928, 123.835242; and the third fish den (iii) at 8.133312, 123.834678,. Although this approach did not involve randomization, it allowed the researchers to gather samples in a practical and consistent manner, taking into account the environmental variability in the region (Nwankwo et al., 2020).

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Figure 2. Fish dens used as sampling points in Malaubang, Ozamiz City, with the following coordinates: (i) 8.130806, 123.834423; (ii) 8.131928, 123.835242; and (iii) 8.133312, 123.834678.

The site consisted of stilt houses and areas affected by pollution point sources due to improper disposal of solid waste that impacted the marine environment and the safety of edible fish caught in the surrounding waters (EMB, 2022). Malaubang is one of the villages in Ozamiz City where significant population growth had been recorded. Ozamiz City have a population of 140,334 residents, with 32,933 households in the 2020 census, projected to grow to 169,302 by 2024 (Philippine Statistics Authority [PSA], 2020). As one of the most consumed foods worldwide, the increasing population emphasized how essential seafood safety and environmental monitoring were due to their direct effects on public health. This opened an avenue for investigating the local issues regarding the ecological condition of the waters of Malaubang and its impact on sustainable food security.

Data Collection

The data collected by the researchers was conducted in accordance with the study's objective to assess microbial contamination and antimicrobial resistance in rabbitfish. Emphasis was placed on ensuring timely acquisition and proper handling of samples to maintain their integrity for microbiological analysis.

Sampling Method

Rabbitfish samples were obtained using convenience sampling, with fish selected based on availability and ease of access. Specimens were collected immediately after being caught by local fishermen, ensuring sample freshness and practical relevance. While this method may limit the statistical representativeness of the findings, it enabled efficient and realistic data collection within the logistical constraints of the study (Alvarado et al., 2021).

Figure 3. Lateral sight of Rabbitfish (*Siganus guttatus*) exhibiting compressed body, dark speckling, long dorsal fin, and bifurcated caudal fin.

Sampling Frequency

A total of 10 rabbitfish (weighing approximately 6 kilograms in total) were collected per sampling event. The sample size was determined using established statistical techniques, considering the anticipated prevalence of *Escherichia coli* and *Salmonella* spp.

to ensure statistical reliability (Akinmoladun et al., 2020). A total of three (3) fish dens were included in the study, with samples taken from a convenient selection based on availability. Samples of about 6kg of fish from the fish dens were obtained and placed directly into separate sterile containers. Each sample consisted of 2 fish, resulting in a total of 5 samples for laboratory analysis.

Season of Sampling

Sample collection was conducted during the dry season; however, on the day of collection—March 4, 2025—Malaubang, Ozamiz City experienced heavy rainfall. This unexpected weather event likely influenced environmental conditions, potentially increasing contamination levels due to sudden runoff and water disturbance. The fish were collected fresh from the fishermen's catch, with the first collection at 6:30 AM and the second at 9:00 AM, aligning with peak fishing activity.

Sample Handling and Preservation

Only the fish meat of the rabbitfish was used for microbiological analysis. To ensure freshness and prevent bacterial contamination, samples fresh from the catch were immediately placed in a sterile ice box with ice packs upon collection. All sampling instruments (knives, containers, gloves, and ice boxes) were sterilized before and after use. Samples were transported to the laboratory within a short period under proper cold-chain conditions to preserve bacterial viability for accurate analysis (Fitting et al., 2020).

Microbial Analysis of Rabbitfish (*Siganus* spp.)

Upon arrival at the laboratory, fish meat samples were subjected to microbiological analysis to detect and enumerate *Escherichia coli* and *Salmonella* spp., using standard culture-based techniques aligned with food safety protocols. The procedure followed methodologies outlined in the FDA's Bacteriological Analytical Manual (BAM) (FDA, October 2020). The method involved homogenizing the fish meat, enriching it in selective broth, and inoculating it onto selective media such as Eosin Methylene Blue (EMB) agar and Salmonella-Shigella Agar (SSA), as detailed in the reference guide (Fitting et al., 2020).

Escherichia coli Analysis

Presumptive Test Escherichia coli

A 50 g portion of rabbitfish meat was aseptically weighed and transferred into a sterile high-speed blender jar, in accordance with FDA compliance protocols for sample size and compositing. Subsequently, 450 mL of Butterfield’s phosphate-buffered diluent was added to the blender jar, and the mixture was blended for 2 minutes to ensure homogenization.

Decimal dilutions were prepared using sterile Butterfield’s phosphate-buffered water. The number of serial dilutions prepared depended on the anticipated coliform density in the samples. All suspensions were mixed by shaking 25 times in a 30 cm arc or vortexed for approximately 7 seconds to ensure homogeneity.

Aliquots of 1 mL from seven serial dilutions were inoculated into Lauryl Sulfate Tryptose (LST) broth tubes for analysis, with five replicates prepared for each dilution. The sixth and seventh dilutions were selected for the subsequent confirmatory testing step, with a total of six tubes utilized from these two dilutions. A 1 mL or 5 mL pipette was used for precise inoculation, ensuring that pipettes were not employed to dispense volumes less than 10% of their total capacity to maintain accuracy. During inoculation, the pipette tip was held at an angle, resting against the inner wall of each tube to minimize contamination and prevent splashing. All dilutions and inoculations were completed within 15 minutes of blending to preserve the viability and integrity of microbial cells.

The inoculated LST broth tubes were incubated at 35°C ± 0.5°C. After 24 ± 2 hours, tubes were examined for gas production, indicated by displacement of the medium in the Durham tube or effervescence upon gentle agitation. Tubes that showed no gas production were re-incubated for an additional 24 hours and re-examined at 48 ± 3 hours. All presumptive positive tubes (gas-producing) were subjected to a confirmed test to validate the presence of *Escherichia coli.*

Confirmed Test for Escherichia coli

From each gas-producing Lauryl Sulfate Tryptose (LST) identified during the presumptive test, a loopful of suspension was aseptically transferred into a tube of Escherichia coli (EC) broth. The EC broth tubes were incubated at 44.5°C for 24 ± 2 hours and subsequently examined for gas production, indicating the presence of fecal coliforms. Tubes that did not show gas formation were re-incubated and re-examined after 48 ± 2 hours.

Completed Test for Escherichia coli

From each gas-producing Lauryl Sulfate Tryptose (LST) or Lactose broth tube identified during the presumptive test, a loopful of suspension was aseptically transferred into a tube of Escherichia coli broth. The EC broth tubes were incubated at 44.5°C for 24 ± 2 hours and subsequently examined for gas production, indicating the presence of fecal coliforms. Tubes that did not show gas formation were re-incubated and re-examined after 24 hours. The identification of *Escherichia coli* was confirmed through a series of biochemical and microbiological tests. Specifically, a colony of *Escherichia coli* was considered positive if at least one of the five isolates tested was identified as *Escherichia coli*. To begin the identification process, Gram staining was performed on all cultures, focusing on those that appeared as Gram-negative, short rods.

Salmonella spp. Analysis

Sample Preparation and Pre-enrichment of Salmonella spp.

A total of 25 grams of fish meat, were aseptically weighed into a sterile blending container. Subsequently, 225 mL of sterile BPW was added, and the mixture was blended for 2 minutes. The homogenized mixture was aseptically transferred into sterile Erlenmeyer (500 mL capacity). The mixtures were then allowed to stand at room temperature for 60 ± 5 minutes with the Erlenmeyer covered with foil and is secured with a rubber band. After standing, the mixtures were well mixed by swirling, the cover were then loosened, and the sample mixtures were incubated for 24 ± 2 hours at 35°C.

Isolation of Salmonella spp.

Following pre-enrichment, the incubated fish meat sample was gently shaken with the lid tightened. From each sample, 0.1 mL of the homogenized mixture was aseptically transferred into 10 mL of Rappaport-Vassiliadis (RV) medium, both enrichment broths were vortexed to ensure thorough mixing. RV medium was incubated at 42 ± 0.2°C for 24 ± 2 hours. After incubation, the broth was mixed again. A 3 mm loopful (approximately 10 µL) of the RV was streaked onto Salmonella-Shigella Agar (SSA) plates. The SSA plates were incubated at 35°C for 24 ± 2 hours.

After incubation, the SSA plates were examined for the presence of colonies exhibiting typical *Salmonella* morphology. On SSA, typical *Salmonella* colonies appeared as colorless or slightly transparent with black centers due to hydrogen sulfide (H_2S) production. In the absence of typical colonies, atypical morphologies such as colorless colonies without black centers or less well-defined black precipitates were also considered for further testing.

From each SSA plate, two or more representative colonies—whether typical or atypical—were carefully selected. The center of each colony was lightly touched with a sterile inoculating needle and used to inoculate both Triple Sugar Iron (TSI) agar and Lysine Iron Agar (LIA) slants. For TSI, the slant was streaked and the butt was stabbed. Without flaming the needle, the same inoculum was used to stab the butt and streak the slant of the LIA tube. LIA slants with a 4 cm deep butt were used to allow anaerobic lysine decarboxylation.

The inoculated TSI and LIA slants were incubated at 35°C for 24 ± 2 hours with caps loosely closed to maintain aerobic conditions and prevent excessive H_2S production. After incubation, the slants were evaluated for reactions characteristic of Salmonella spp.

In TSI agar, *Salmonella* spp. typically produced an alkaline (red) slant and acid (yellow) butt, often with blackening from H_2S production. In LIA, an alkaline (purple) butt was considered typical. Cultures exhibiting an acid (yellow) butt in LIA or no change in the TSI slant and butt were considered atypical and were either discarded or re-evaluated based on colony origin and morphology. All results were interpreted according to standardized bacteriological guidelines, and results were tabulated in summary tables to determine organism presence in replicates. This process of identification verified target organism identity and the validity of preliminary presumptive culture results.

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

Colony-forming units (CFU) per gram of rabbitfish flesh were determined from numbers on Eosin Methylene Blue (EMB) agar for *Escherichia coli* and Salmonella - Shigella Agar (SSA) for *Salmonella* spp... Using the standard CFU formula:
First get the average colony count,

$$\text{Average Colony Count} = \frac{Sum\ of\ all\ colony\ counts}{Number\ of\ positive\ replicates}$$

Then,

$$\text{CFU/g} = \frac{Number\ of\ colonies\ x\ Dilution\ factor}{Volume\ of\ culture\ plated\ (mL)}$$

This permitted the quantitative determination of microbial load in individual fish samples. The findings were contrasted with microbiological limits specified by food safety authorities, such as the International Commission on Microbiological Specifications for Foods (ICMSF) and the World Health Organization (WHO) food safety authorities.

Antimicrobial Drug Susceptibility Testing

The antimicrobial susceptibility profiles of *Escherichia coli* and *Salmonella* spp. isolates were determined using the Kirby–Bauer disc diffusion method on Mueller Hinton Agar (MHA) (Oxoid, Basingstoke, England), following the guidelines of the Clinical Laboratory Standards Institute (Ava et al., 2020). The isolates were tested against commercially available antibiotic discs (HiMedia), which included ampicillin (2 µg), gentamicin (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg). These antibiotics were selected based on their availability and in accordance with the recommendations from the World Health Organization (WHO) and the World Organization for Animal Health (WOAH/OIE) for both human and veterinary use (World Organisation for Animal Health, 2021).

The test organisms were uniformly seeded onto the surface of MHA plates and subsequently exposed to the antibiotic discs. Plates were incubated at 37 °C for 24 hours. The diameters of the zones of inhibition around each disc were measured in centimeters, and the isolates were classified as susceptible, intermediate, or resistant based on Clinical Laboratory Standards Institute interpretative standards. Isolates exhibiting resistance to at least one agent in three or more antimicrobial classes were classified as multidrug-resistant

(MDR), in line with standard definitions (Clinical and Laboratory Standards Institute, 2025).

Zones of inhibition were initially measured in centimeters and then converted to millimeters by multiplying each value by 10, in accordance with standardized reporting units. For each sample, the average zone diameter was calculated by averaging the measurements from two replicates per sample. The resulting average zone diameters from the replicates were then interpreted based on the Clinical and Laboratory Standards Institute (CLSI, 2025) breakpoints.

After interpreting the zone diameters, the resistance rates were calculated using the formula:

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

Data Analysis

Quantitative and descriptive information acquired through microbial isolation, colony counting, biochemical testing, and antimicrobial susceptibility profiling were used to examine the contamination of *Escherichia coli* and *Salmonella* spp., in rabbitfish (*Siganus guttatus*) sampled from Malaubang, Ozamiz City.

Microbiological Enumeration

To quantify the microbial load, colony counts from culture plates were used to compute the colony forming units per gram (CFU/g) after getting the average using the formula:

$$\text{CFU/g} = \frac{Number\ of\ colonies\ x\ Dilution\ factor}{Volume\ of\ culture\ plated\ (mL)}$$

To standardize the data, the values were converted to their logarithmic form ($\log_{10}$ CFU/g). For instance, in one replicate, 371 colonies were observed from a 10^7 dilution with 0.1 mL plated. Applying the formula:

$$\text{CFU/g} = \frac{371\ x\ 10^{10}}{0.1} = 3.71\ x\ 10^{10}$$

$$\log_{10}\text{CFU/g} = \log_{10}(3.71\times10^{10}) \approx 10.57$$

This permitted the quantitative determination of microbial load in individual fish samples. The findings were contrasted with microbiological limits specified by food safety authorities, such as the International Commission on Microbiological Specifications for Foods (ICMSF) and the World Health Organization (WHO) food safety authorities.

Antimicrobial Susceptibility Testing (AST)

Each fish tissue sample underwent duplicate disk diffusion testing using five antibiotics: ampicillin (AMP), ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (CN), and chloramphenicol (C). The diameters of the inhibition zones (in mm) were measured for each replicate. The two values per antibiotic were averaged to obtain a representative zone diameter per sample. These averages were interpreted according to the 2025 CLSI guidelines to classify bacterial isolates as Susceptible (S), Intermediate (I), or Resistant (R). A conservative interpretation was used—if one replicate showed resistance, the sample was marked resistant. Finally, the percentage resistance for each antibiotic was calculated using the formula:

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

For example, for Ampicillin (AMP), all 5 out of 5 samples showed resistance, resulting in (5 ÷ 5) × 100 = 100% resistance. This process was applied across all tested antibiotics to assess their effectiveness against the bacterial isolates.

Spatial and Environmental Relevance

Although inferential statistics were not employed, the use of convenience sampling provided practical access to fish samples across the fishing site. While this approach limited statistical generalizability, it still offered insights into contamination trends within accessible areas. The study was conducted during the dry season; however, episodes of heavy rainfall likely contributed to increased microbial contamination due to environmental runoff. These conditions suggest a potential link between sudden rain events and the transport of pollutants from nearby residential or waste discharge areas into the aquatic environment.

Biosafety, Biosecurity, and Biowaste Management

In this study, all microbiological procedures were conducted in a Biosafety Cabinet (BSC) Level II at the Microbiology Laboratory Maria Mercado Hall under Biosafety Level 2 (BSL-2) conditions. Complete personal protective equipment (PPE), such as lab coats, face masks, and gloves, was used by all student researchers to guarantee safety and avoid contamination (Section 3.1, FDA, 2019). Glass stirring rods, test tubes, and Erlenmeyer flasks were all autoclaved at 121°C for 15 minutes before to and following use, and disposable Petri dishes and inoculating loops were utilized to reduce cross-contamination (Section 3.2, FDA, 2019). All contaminated materials, such as spent agar plates, inoculating equipment, and disposable items, were autoclaved during microbial culture to

guarantee total bacterial inactivation (Section 3.3, FDA, 2019). Biohazardous waste was managed and disposed of in accordance with the institution's internal protocols, which are in compliance with national standards and the FDA's biosafety and waste disposal regulations (FDA, 2019, Section 3.3). Work surfaces and reusable tools were decontaminated using the proper disinfectants. The Microbiological Methods Validation Guidelines were closely adhered to in every operation to provide a sterile and secure environment for the duration of the investigation.

Sample Treatment

The integrity of the rabbitfish (*Siganus guttatus*) specimens and the precision of the microbiological analyses were guaranteed for this investigation by adhering to the correct sample treatment protocols. The specimens were handled by accepted dissection practices to prevent cross-contamination and maintain sample quality to avoid external contamination. Sterile tools were used to dissect each rabbitfish, and a sterile knife was used to separate the fish meat meticulously to guarantee representativeness; the meat from various areas of each species was thoroughly sampled, and all dissected materials were processed under sterile conditions.

The fish samples were stored in sterile containers to preserve their microbiological integrity. Subsequently, these containers were put inside an icebox filled with ice packs to maintain the samples' constant low temperature. By keeping the specimens stable throughout transit to the lab, the icebox reduced the growth of germs and preserved the samples' microbiological composition.

Gloves were worn during the sample collection procedure to prevent contamination and maintain good hand hygiene to avoid cross-contamination between specimens; all tools used for dissection and sample collection were sanitized both before and after each use. The dissecting area was carefully cleansed and disinfected to reduce the possibility of sample contamination.

Ethical Consideration

To protect all parties involved—local fishermen, the people of Malaubang, Ozamiz City, and the maritime environment from which rabbitfish (*Siganus guttatus*) samples were taken—this study complied with ethical guidelines. Important information regarding the fishing sites and rabbitfish occurrence in the area was obtained by consulting local fishermen. Before any conversations or interviews, their informed consent was acquired, and their involvement was voluntary. They received a thorough explanation of the study's goals and purpose, and their identities and private data were kept confidential. The study also put the inhabitants' welfare first by carrying out sampling operations that caused the least disturbance to their regular schedules and fishing activities.

Additionally, rabbitfish specimens were harvested responsibly to minimize overexploitation of the species and unnecessary damage to the marine habitat. Only the required samples were taken, and all specimens were treated carefully according to accepted laboratory procedures. By considering these ethical issues, the study ensured that it was carried out ethically and with respect for all individuals involved.

RESULTS AND DISCUSSION

Quantification of Bacterial Contamination

The microbiological analysis of rabbitfish (*Siganus guttatus*) samples collected from Malaubang, Ozamiz City, revealed substantial levels of bacterial contamination. Both total aerobic bacterial counts and *Escherichia coli* levels were quantified to assess the microbial quality and safety of the fish meat. All five samples showed significantly high bacterial loads, with *Escherichia coli* counts ranging from 2.96×10^8 to 3.61×10^{10} CFU/g and total aerobic bacteria from 3.41×10^9 to 4.34×10^{10} CFU/g. In terms of logarithmic values, the samples exhibited $\log_{10}$ CFU/g levels between 9.47 and 10.56, indicating widespread and substantial bacterial presence.

Sample 2 recorded the highest contamination levels among all specimens, surpassing 10^{10} CFU/g for both *Escherichia coli* and total bacteria, which may reflect acute microbial exposure potentially linked to recent contamination events. These elevated values exceed the acceptable microbiological thresholds for fresh fish as recommended by international food safety authorities, such as the International Commission on Microbiological Specifications for Foods (ICMSF, 2020), highlighting potential public health risks. These findings align with previous studies conducted in similarly polluted coastal environments, where poor sanitation and ineffective waste management systems were identified as major contributors to bacterial contamination in marine organisms (Faridullah et al., 2022; Roongrojmongkhon et al., 2022).

Table 1. Bacterial Counts of *Escherichia coli* and Total Bacteria in Rabbitfish Samples

	Colony-Forming Unit per gram (CFU/g) of Escherichia coli Isolated on EMB Agar (Selective)		Colony-Forming Unit per gram (CFU/g) of Total Bacteria on PCA (General Plate Count)	
Sample	**CFU/g**	**$\log_{10}$ CFU/g**	**CFU/g**	**$\log_{10}$ CFU/g**
Sample 1	1.15×10^{10}	10.06	1.73×10^{10}	10.24
Sample 2	3.61×10^{10}	10.56	4.34×10^{10}	10.64
Sample 3	3.19×10^{10}	10.50	3.09×10^{10}	10.49
Sample 4	3.03×10^{8}	9.48	4.89×10^{9}	9.69
Sample 5	2.96×10^{8}	9.47	3.41×10^{9}	9.53

Note: Values are expressed as colony-forming units per gram (CFU/g) and $\log_{10}$ CFU/g.

The consistent presence of *Escherichia coli,* a known fecal indicator organism, in all fish samples confirms ongoing sanitary issues in the area and emphasizes the urgent need for improved environmental and waste management strategies in Malaubang. As presented in Table 1, the detailed breakdown of bacterial counts per sample.

Similar to the *Escherichia coli* and *Salmonella* spp. were detected in all five rabbitfish samples, with CFU counts ranging from 9.89×10^{8} to 2.84×10^{10} CFU/g. The highest contamination was observed in Sample 3, while Sample 5 had the lowest. These findings mirror the trends seen with *Escherichia coli* and suggest common contamination pathways likely related to human and animal waste in the aquatic environment. Table 2 provided a comprehensive summary of the bacterial loads per sample.

Table 2. Bacterial Counts of *Salmonella* spp. in Rabbitfish Samples

Sample	CFU/g	$\log_{10}$ CFU/g
Sample 1	1.08×10^{10}	10.03
Sample 2	2.74×10^{10}	10.44
Sample 3	2.84×10^{10}	10.45
Sample 4	2.75×10^{9}	9.44
Sample 5	9.89×10^{8}	9.00

Note: Values are expressed as colony-forming units per gram (CFU/g) and $\log_{10}$ CFU/g.

The levels again surpass the acceptable limits set by food safety authorities, highlighting the widespread contamination. The detection of *Salmonella* spp. is concerning due to its potential to cause severe gastrointestinal illness, especially when seafood is consumed raw or undercooked. Comparable studies in coastal environments similarly report high prevalence rates of *Salmonella* spp. in seafood products due to contaminated aquatic environments (Yoon et al., 2021; Boutaib et al., 2020). These findings emphasize the urgent need for effective sanitation, proper wastewater treatment, and monitoring of seafood safety in fishing communities.

Biochemical Test for Confirmation

The isolates that grew on Eosin Methylene Blue (EMB) agar and exhibited the characteristic green metallic sheen were subjected to Gram staining for confirmatory identification. All samples yielded Gram-negative rods under microscopic examination, which is consistent with the typical morphology of *Escherichia coli*. The universal presence of these organisms reinforces the findings from Table 1 and supports the biochemical identification of the isolates. The uniform morphology of Escherichia coli observed in the Gram stain analysis, as seen in Table 3, further reinforces the potential link to fecal contamination and the associated risks to public health.

Table 3. Gram Stain Results and Morphology of Rabbitfish Samples

Sample	Gram Stain Result	Morphology Observed
Sample 1	Positive	Gram-negative Rods (Bacilli)
Sample 2	Positive	Gram-negative Rods (Bacilli)
Sample 3	Positive	Gram-negative Rods (Bacilli)
Sample 4	Positive	Gram-negative Rods (Bacilli)
Sample 5	Positive	Gram-negative Rods (Bacilli)

The consistent results of the biochemical test needed to confirm *Escherichia coli* across samples suggest widespread presence of this bacteria in the rabbitfish collected from Malaubang, most likely linked to fecal contamination of the aquatic environment. This highlights the potential exposure of marine organisms to untreated domestic waste, particularly in densely populated or poorly regulated coastal areas, and reinforces the need for environmental sanitation and food safety monitoring.

The identification was confirmed by the production of hydrogen sulfide (H_2S) on TSI and LIA slants and the appearance of black-centered colonies on SSA. All isolates yielded characteristic reactions for *Salmonella* spp. in both TSI and LIA media, including hydrogen sulfide production and typical color changes. Specifically, reactions such as A/A with H_2S production in TSI and K/K with H_2S in LIA were consistent across all samples, confirming the presence of *Salmonella* spp. These biochemical confirmations align with colony morphology observed on Salmonella-Shigella agar, they appeared colorless with black centers, attributed to hydrogen sulfide production. These biochemical reactions aligned with *Salmonella* spp. characteristics, affirms the reliability of presumptive identifications and supporting the conclusion of widespread *Salmonella* contamination.

The consistent presence of *Salmonella* spp. across all five rabbitfish samples highlights the environmental risk posed by inadequate waste management in the area, particularly the release of untreated domestic and industrial waste into the surrounding waters. The presence of *Salmonella* spp. is particularly alarming, as it poses a severe risk for foodborne illness, especially in raw or undercooked seafood.

Table 4. Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) Reactions of *Salmonella* spp. in Rabbitfish Samples

Sample	TSI Result	G+	LIA Result	G+	Identification
Sample 1	A/A, H_2S^+	+	K/K, H_2S^+	+	*Salmonella* spp.
Sample 2	A/A, H_2S^+	+	K/K, H_2S^+	+	*Salmonella* spp.
Sample 3	A/A, H_2S^+	+	K/K, H_2S^+	+	*Salmonella* spp.
Sample 4	A/A, H_2S^+	+	K/K, H_2S^+	+	*Salmonella* spp.
Sample 5	A/A, H_2S^+	+	K/K, H_2S^+	+	*Salmonella* spp.

Note: In TSI (Triple Sugar Iron) agar, A = acid (yellow), K = alkaline (red), H_2S^+ = hydrogen sulfide production (black precipitate), and G^+ = gas production, while in LIA (Lysine Iron Agar), A = acid (yellow), K = alkaline (purple), H_2S^+ = hydrogen sulfide production (black precipitate), and G^+ = gas production, with each medium testing different metabolic pathways.

The consistent presence of Salmonella spp. across all five rabbitfish samples highlights the environmental risk posed by inadequate waste management in the area, particularly the release of untreated domestic and industrial waste into the surrounding waters. The presence of *Salmonella* spp. is particularly alarming, as it poses a severe risk for foodborne illness, especially in raw or undercooked seafood. Similar contamination levels have been observed in coastal seafood in other studies (Yoon et al., 2021; Boutaib et al., 2020), and were often linked to polluted water sources near densely populated or industrialized zones. Table 4 presented the consistent biochemical reactions across all samples, including the typical TSI and LIA results, confirm the presence of *Salmonella* spp.

Antibiotic Resistance Profiles

The antibiotic susceptibility testing (AST) of *Escherichia coli* isolates obtained from Rabbitfish (*Siganus guttatus*) revealed varying levels of resistance and intermediate

susceptibility across five commonly used antibiotics, as interpreted using the Clinical and Laboratory Standards Institute (CLSI) 2025 standards.

As shown in Table 5, all four isolates exhibited resistance to ampicillin (AMP), with inhibition zone diameters ranging from 11.0 mm to 18.0 mm. For ceftriaxone (CRO), two isolates demonstrated intermediate susceptibility (26.0 mm and 24.0 mm), one isolate was susceptible (19.5 mm), and one isolate also showed intermediate susceptibility (14.0 mm). Ciprofloxacin (CIP) showed mostly effective results, with three isolates classified as susceptible and one as intermediate, although the observed inhibition zones ranged only from 2.5 mm to 7.0 mm. Gentamicin (CN) displayed poor efficacy, as all isolates were resistant with zone diameters between 3.5 mm and 10.0 mm. For chloramphenicol (C), three isolates were resistant, and one isolate showed intermediate susceptibility.

Table 5. Average Zone Diameters and Clinical & Laboratory Standards Institute 2025 Interpretation of *Escherichia coli*

Sample	Ampicillin (AMP)	Ceftriaxone (CRO)	Ciprofloxacin (CIP)	Gentamicin (CN)	Chloramphenicol (C)
Sample 1	11.0 (R)	26.0 (I)	7.0 (S)	4.5 (R)	R (R)
Sample 2	18.0 (R)	19.5 (S)	2.5 (S)	7.5 (R)	R (R)
Sample 3	15.0 (R)	24.0 (I)	7.0 (S)	10.0 (R)	I (I)
Sample 4	13.0 (R)	14.0 (I)	4.0 (I)	3.5 (R)	R (R)
Sample 5	17.5 (R)	20.5 (S)	3.5 (S)	11.5 (R)	S (S)

Note: R – Resistant, I – Intermediate, S – Susceptible; Zone diameters are in millimeters (mm).

The consistent resistance of all *Escherichia coli* isolates to ampicillin (AMP) aligned with the findings of Ava et al. (2020) and Gordon et al. (2021), who reported widespread resistance to β-lactam antibiotics in aquatic *Escherichia coli* isolates. This could be attributed to the historical overuse of such antibiotics in aquaculture and human medicine, leading to selective pressure and resistance development.

Mixed susceptibility patterns observed with ceftriaxone (CRO), an extended-spectrum cephalosporin, suggested reduced antibiotic efficacy. Although complete resistance was not evident, the intermediate susceptibility of three out of four isolates raised concern over emerging tolerance, possibly due to horizontal gene transfer of β-lactamase genes within aquatic microbial communities, as supported by Oliveira et al. (2021) and Delgado et al. (2021).

Ciprofloxacin (CIP) retained some degree of effectiveness, with three isolates susceptible and one intermediate. However, the small inhibition zones (2.5 mm to 7.0 mm) indicated potential early resistance development, possibly through chromosomal mutations in genes such as gyrA or parC, consistent with observations by Jian et al. (2021).

Gentamicin (CN) was ineffective across all isolates, which were classified as resistant. This supported the findings of Mumbo et al. (2023), who noted high aminoglycoside resistance among *Escherichia coli* from fish intended for human consumption. The resistance was likely facilitated by mobile genetic elements such as integrons and plasmids carrying aminoglycoside-modifying enzyme genes, as described by Mancuso et al. (2021).

For chloramphenicol (C), the predominance of resistant isolates—with only one showing intermediate susceptibility—reflected the persistence of resistance in aquatic environments. Despite the restricted use of this antibiotic in food-producing animals, previous studies by Martins et al. (2019) and Shen et al. (2020) similarly reported resistance, suggesting legacy effects of past misuse and ongoing dissemination of resistance determinants in the environment.

The antibiotic susceptibility testing (AST) of *Salmonella* spp. isolated from Rabbitfish (*Siganus* spp.) was conducted using five antibiotics: ampicillin (AMP), ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (CN), and chloramphenicol (C), in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2025 guidelines.

As presented in Table 6, all five *Salmonella* spp. isolates exhibited resistance to ampicillin, with inhibition zone diameters ranging from 8.5 mm to 17.0 mm. Ceftriaxone displayed variable results, with Samples 1 and 3 classified as susceptible, Sample 4 as intermediate, and Samples 2 and 5 as resistant. Ciprofloxacin showed universal susceptibility among all isolates, despite some zone diameters being relatively small (e.g., Sample 2 at 3.0 mm and Sample 4 at 3.5 mm). All isolates were resistant to gentamicin, with inhibition zones ranging from 1.0 mm to 11.0 mm. For chloramphenicol, susceptibility patterns were mixed: Samples 3 and 4 were susceptible, while Samples 1, 2, and 5 showed resistance or intermediate susceptibility.

Table 6. Average Zone Diameters and Clinical & Laboratory Standards Institute 2025 Interpretation of *Salmonella* spp.

Sample	Ampicillin (AMP)	Ceftriaxone (CRO)	Ciprofloxacin (CIP)	Gentamicin (CN)	Chloramphenicol (C)
Sample 1	15.0 (R)	18.0 (S)	4.5 (S)	1.0 (R)	R (R)
Sample 2	8.5 (R)	18.0 (R)	3.0 (S)	5.0 (R)	R (R)
Sample 3	17.0 (R)	23.0 (S)	7.0 (S)	11.0 (R)	S (S)
Sample 4	12.5 (R)	18.5 (I)	3.5 (S)	11.0 (R)	S (S)
Sample 5	10.5 (R)	20.0 (R)	5.5 (S)	9.5 (R)	I (I)

Note: R – Resistant, I – Intermediate, S – Susceptible; Zone diameters are in millimeters (mm).

The universal resistance to ampicillin among the *Salmonella* spp. isolates corroborated the findings of Shen et al. (2020), who reported widespread beta-lactam resistance among aquatic pathogens. This pattern may be attributed to the prolonged use of

penicillin-type antibiotics in aquaculture, contributing to the development and persistence of resistant strains.

Ceftriaxone, a third-generation cephalosporin, demonstrated inconsistent efficacy. While some isolates were susceptible, others displayed resistance or intermediate responses. These results suggested the possible presence of extended-spectrum beta-lactamase (ESBL)-producing Salmonella, as noted in studies by Roongrojmongkhon et al. (2022) and Jian et al. (2021), which observed sporadic cephalosporin resistance in marine bacterial communities.

Despite low inhibition zone diameters in some samples, ciprofloxacin retained full efficacy across all isolates. This universal susceptibility reaffirmed its effectiveness against Salmonella spp., consistent with the findings of Su et al. (2020) and Gordon et al. (2021), who highlighted fluoroquinolones as reliable agents for treating enteric pathogens from aquatic sources.

All isolates showed resistance to gentamicin, which may be attributed to the presence of aminoglycoside-modifying enzymes or active efflux mechanisms, as proposed by Oliveira et al. (2021). The persistence of this resistance is concerning, particularly given gentamicin's common usage in aquaculture, as discussed by Mhongole and Mdegela (2022).

The results for chloramphenicol were mixed. While two isolates were susceptible, the others demonstrated resistance or intermediate responses. This pattern may reflect differences in environmental exposure or residual contamination, as previously documented by Oleastro et al. (2022) and Park et al. (2020). The variability in susceptibility

could also be influenced by ongoing regulatory restrictions on chloramphenicol use in food animals and aquatic systems.

All *Escherichia coli* isolates exhibited complete (100%) resistance to both ampicillin and gentamicin, indicating high levels of resistance to both first-line β-lactam and aminoglycoside antibiotics. In addition, 80% of the isolates were resistant to chloramphenicol, while 40% showed resistance to ceftriaxone. Ciprofloxacin was the only antibiotic to which all Escherichia coli isolates remained fully susceptible, showing 0% resistance.

Similarly, all *Salmonella* spp. isolates showed 100% resistance to ampicillin and gentamicin, mirroring the resistance profile observed in *Escherichia coli* resistance to chloramphenicol and ceftriaxone was detected in 40% of the Salmonella isolates. Ciprofloxacin demonstrated full efficacy across all samples, with 0% resistance reported. As summarized in Table 7, both *Escherichia coli* and *Salmonella* spp. exhibited multidrug-resistant (MDR) profiles, with resistance to three or more classes of antibiotics.

Table 7. Antibiotic Resistance Patterns of *Escherichia coli* and *Salmonella* spp.

Antibiotic	*Escherichia coli* Resistant (%)	*Escherichia coli* Interpretation	*Salmonella* spp. Resistant (%)	*Salmonella* spp, Interpretation
Ampicillin (AMP)	100%	High Resistance	100%	High Resistance
Ceftriaxone (CRO)	40%	Moderately Resistant	40%	Moderately Resistant
Ciprofloxacin (CIP)	0%	Fully Sensitive	0%	Fully Sensitive
Gentamicin (CN)	100%	High Resistance	100%	High Resistance
Chloramphenicol (C)	80%	Moderate to High Resistance	40%	Moderate Resistance

Note: Resistance interpretation is based on the percentage of resistant isolates, where 0–20% is considered Fully Sensitive, 21–40% Moderately Resistant, 41–60% Intermediate Resistance, 61–80% Moderate to High Resistance, and 81–100% High Resistance.

The complete resistance of *Escherichia coli* and *Salmonella* spp. to ampicillin and gentamicin reflected widespread antimicrobial resistance to both commonly used first-line and aminoglycoside antibiotics. This pattern indicated that these bacterial strains possess robust resistance mechanisms, potentially driven by environmental exposure to selective pressures from excessive antibiotic use.

The observed 80% resistance to chloramphenicol in *Escherichia coli* and 40% resistance in *Salmonella* spp. suggested that, despite regulatory controls, legacy use and persistent environmental contamination continue to influence susceptibility patterns. Similarly, the moderate resistance to ceftriaxone in both bacterial species (40%) may be indicative of emerging extended-spectrum β-lactamase (ESBL) activity, which warrants further molecular investigation.

Ciprofloxacin's complete efficacy against both *Escherichia coli* and *Salmonella* spp. isolates was noteworthy and consistent with global reports emphasizing the continued reliability of fluoroquinolones for treating enteric infections. However, the emergence of resistance to other antibiotic classes signals the need for cautious use to preserve ciprofloxacin’s effectiveness.

The detection of multidrug-resistant (MDR) *Escherichia coli* and *Salmonella* spp. in Rabbitfish (*Siganus guttatus*), a fish frequently consumed in the local community, underscored potential public health risks. The presence of MDR strains in seafood suggested a route of transmission for antimicrobial-resistant pathogens to humans through the food chain.

These findings supported earlier research (Gordon et al., 2021; Delgado et al., 2021) that linked the rise of antimicrobial resistance (AMR) in aquatic environments to the overuse of antibiotics in aquaculture and the release of untreated agricultural and domestic waste into marine ecosystems. Such environmental practices promote horizontal gene transfer and the dissemination of resistance genes, thereby amplifying the public health threat posed by resistant pathogens in coastal regions.

The microbiological assessment of rabbitfish (*Siganus guttatus*) from Malaubang revealed significant contamination with *Escherichia coli* and *Salmonella* spp., both exceeding internationally accepted food safety thresholds. Across all five samples, the total bacterial load, *Escherichia coli* and *Salmonella* spp. counts consistently surpassed 10^8 CFU/g, indicating severe microbial contamination. Sample 2 showed the highest total bacterial and *Escherichia coli* counts (4.34×10^{10} and 3.61×10^{10} CFU/g, respectively), while Sample 3 recorded the highest *Salmonella* spp. count (2.84×10^{10} CFU/g), as shown in Tables 1 and 2. These levels greatly exceed acceptable microbial limits for fish (ICMSF, 2020) and point to probable fecal contamination, likely due to poor sanitation, wastewater runoff, or unmanaged human and animal waste.

Morphological and biochemical identification reinforced the presence of these pathogens. All isolates grown on EMB agar exhibited the characteristic green metallic sheen of *Escherichia coli* and were confirmed as Gram-negative bacilli under microscopy (Table 3). Similarly, *Salmonella* spp. were biochemically confirmed through typical hydrogen sulfide production on TSI and LIA media, alongside gas production and color changes consistent with known *Salmonella* profiles (Table 4). The consistent reactions across all samples indicated reliable biochemical confirmation and a uniform pathogenic

profile, suggesting a common source of contamination—possibly untreated domestic waste entering coastal waters.

When comparing the occurrence and distribution of both pathogens, *Escherichia coli* was slightly more prevalent in terms of bacterial load, particularly in Sample 2. However, *Salmonella* spp. counts in Sample 3 were comparable, suggesting that both bacteria are endemic to the aquatic environment in Malaubang and may share contamination pathways. These findings align with similar studies in coastal communities where proximity to urban runoff and lack of proper sewage treatment have led to high seafood contamination (Faridullah et al., 2022; Yoon et al., 2021).

Antibiotic susceptibility testing further revealed multidrug resistance in both bacterial species. *Escherichia coli* isolates exhibited complete resistance (100%) to ampicillin and gentamicin, while ciprofloxacin remained the only consistently effective antibiotic (Table 5). Intermediate or partial resistance was observed with ceftriaxone and chloramphenicol. This pattern suggests historical overuse or environmental persistence of β-lactam and aminoglycoside antibiotics, potentially driving resistance via plasmid-encoded resistance genes or integrons (Mumbo et al., 2023; Mancuso et al., 2021).

Similar pattern of resistance in *Salmonella* spp. (Table 6), particularly to ampicillin, but relatively lower resistance levels to gentamicin and chloramphenicol compared to *Escherichia coli.* This differential susceptibility may indicate varying exposure to antimicrobials or intrinsic resistance mechanisms between species. Nonetheless, both bacteria demonstrated ciprofloxacin susceptibility, making it a potential treatment option, though caution is warranted given the early signs of resistance emergence (Jian et al., 2021).

CONCLUSION AND RECOMMENDATION

This study confirmed the presence of *Escherichia coli* and *Salmonella* spp. in the meat of rabbitfish (*Siganus guttatus*) collected from Malaubang, Ozamiz City. Bacterial loads were high, and multiple isolates exhibited multidrug resistance. These results indicate significant microbiological contamination and highlight a potential public health threat. Immediate improvements in sanitation, waste management, and seafood safety practices in the area are necessary to reduce future risks.

To broaden the scope of this study, future researchers are encouraged to include comprehensive water quality assessments in the sampling sites of Malaubang, Ozamiz City, particularly Boulevard New Road. This would involve analyzing both physicochemical and microbiological properties of the coastal water, which may yield crucial data supporting the bacterial contamination observed in rabbitfish (*Siganus guttatus*). These findings may be further substantiated by data from credible agencies such as the Department of Environment and Natural Resources (DENR), the Environmental Management Bureau (EMB), or local water quality monitoring initiatives. Utilizing data from reliable sources will ensure that subsequent investigations are grounded in evidence-based environmental assessments and avoid misinterpretations that may hinder accurate conclusions.

Additionally, researchers should consider integrating environmental data with anthropogenic variables such as coastal population density, sanitation access, and waste disposal practices to determine contributing factors to microbial pollution. Expert

perspectives from marine biologists, environmental scientists, and public health authorities may also strengthen the study by offering deeper insight into the ecological and health implications of coastal contamination.

Moreover, it is recommended that local policymakers strengthen waste management and sanitation programs in coastal areas. This could involve community-based waste reduction initiatives, stricter enforcement of waste discharge regulations, and infrastructure improvements such as establishing proper drainage and sewage treatment systems. These steps are essential in reducing the entry of fecal matter and other contaminants into marine waters and preventing the recurrence of bacterial outbreaks in seafood.

Lastly, increasing public awareness of seafood safety must be a key component of local health strategies. Emphasis should also be placed on the risks of consuming raw or undercooked fish and the importance of proper cooking techniques to eliminate foodborne pathogens. These efforts, if sustained, will not only protect public health but also contribute to the long-term sustainability of marine resources and food security in the community.

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APPENDICES

APPENDIX A. Computations

Replicate	Colonies	Dilution	Volume Plated (mL)	CFU/g	$\log_{10}$ CFU/g
1.0	371	7	0.1	3.71E+10	10.56937
1.1	127	7	0.1	1.27E+10	10.1038
2.1	326	7	0.1	3.26E+10	10.51322
2.2	395	7	0.1	3.95E+10	10.5966
3.0	319	7	0.1	3.19E+10	10.50379
4.2.1	421	7	0.1	4.21E+10	10.62428
4.2.2	254	6	0.1	2.54E+09	9.404834
4.2.3	291	6	0.1	2.91E+09	9.463893
4.2.4	211	6	0.1	2.11E+09	9.324282
4.2.5	170	6	0.1	1.7E+09	9.230449
5.0	328	6	0.1	3.28E+09	9.515874
5.1	263	6	0.1	2.63E+09	9.419956

A.1. Computed CFU/g and $\log_{10}$ CFU/g of *Escherichia coli* Isolated from EMB Agar

Replicate	Colonies	Dilution	Volume Plated (mL)	CFU/g	$\log_{10}$ CFU/g
1.0	157	7	0.1	1.57E+10	10.1959
1.1	143	7	0.1	1.43E+10	10.15534
1.2	220	7	0.1	2.2E+10	10.34242
2.1	178	7	0.1	1.78E+10	10.25042
2.2	902	7	0.1	9.02E+10	10.95521
2.3	222	7	0.1	2.22E+10	10.34635
3.0	309	7	0.1	3.09E+10	10.48996
4.0	335	7	0.1	3.35E+10	10.52504
4.2.1	387	6	0.1	3.87E+09	9.587711
4.2.2	706	6	0.1	7.06E+09	9.848805
4.2.3	538	6	0.1	5.38E+09	9.730782
4.2.4	324	6	0.1	3.24E+09	9.510545
5.0	321	6	0.1	3.21E+09	9.506505
5.1	208	6	0.1	2.08E+09	9.318063
5.2	516	6	0.1	5.16E+09	9.71265
5.3	318	6	0.1	3.18E+09	9.502427

A.2. Computed CFU/g and $\log_{10}$ CFU/g of *Escherichia coli* from PCA Agar – Confirmatory

APPENDIX A. Cont'd

Replicate	Colonies	Dilution	Volume Plated (mL)	CFU/g	log_{10} CFU/g
1.3	108	7	0.1	1.08E+10	10.03342
2.1	264	7	0.1	2.64E+10	10.4216
2.5	284	7	0.1	2.84E+10	10.45332
3.2	136	7	0.1	1.36E+10	10.13354
3.3	306	7	0.1	3.06E+10	10.48572
3.4	411	7	0.1	4.11E+10	10.61384
4.2	17	7	0.1	1.7E+09	9.230449
4.4	38	7	0.1	3.8E+09	9.579784
4.5	426	6	0.1	4.26E+09	9.62941
5.1	85	6	0.1	8.5E+08	8.929419
5.1.0	51	6	0.1	5.1E+08	8.70757
5.2	181	6	0.1	1.81E+09	9.257679
5.2.1	79	6	0.1	7.9E+08	8.897627
5.3.0	236	6	0.1	2.36E+09	9.372912
5.4	47	6	0.1	4.7E+08	8.672098
5.4.1	73	6	0.1	7.3E+08	8.863323
5.5	33	6	0.1	3.3E+08	8.518514
5.5.0	105	6	0.1	1.05E+09	9.021189

A.3. Computed CFU/g and log_{10} CFU/g of *Salmonella* spp. Isolated from SSA Agar

APPENDIX A. Cont'd

Replicate	AMP	CRO	CIP	CN	C
1.1	R	12 mm	26 mm	9 mm	6 mm
1.2	R	10 mm	26 mm	5 mm	3 mm
Avg (Sample 1)	**11 mm** (=(12+10)/2)	**26 mm**	**7 mm**	**4.5 mm**	**R** (since one is R)
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
2.3	R	20 mm	21 mm	2 mm	11 mm
2.4	R	16 mm	18 mm	3 mm	4 mm
Avg (Sample 2)	**18 mm**	**19.5 mm**	**2.5 mm**	**7.5 mm**	**R** (one value is R)
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
3.5	R	19 mm	24 mm	7 mm	11 mm
3.6	R	11 mm	24 mm	7 mm	9 mm
Avg (Sample 3)	**15 mm**	**24 mm**	**7 mm**	**10 mm**	**I**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
4.1	R	17 mm	17 mm	2 mm	3 mm
4.2	R	9 mm	11 mm	6 mm	4 mm
Avg (Sample 4)	**13 mm**	**14 mm**	**4 mm**	**3.5 mm**	**R**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
5.3	2 mm	23 mm	21 mm	5 mm	19 mm
5.4	R	12 mm	20 mm	2 mm	4 mm
Avg (Sample 5)	**17.5 mm** (=(20+15)/2, using 20mm for 2.0cm and ~15 for R)	**20.5 mm**	**3.5 mm**	**11.5 mm**	**S**

Legend: AMP (Ampicillin), CRO (Ceftriaxone), CIP (Ciprofloxacin), CN (Gentamicin), and C (Chloramphenicol) were tested. Interpretations follow CLSI 2025: S = Susceptible, I = Intermediate, R = Resistant.

A.4. Antibiotic Susceptibility Results for Escherichia coli: Raw and Averaged Zone Diameters with CLSI Interpretation

APPENDIX A. Cont'd

Replicate	AMP	CRO	CIP	CN	C
1.1	R	18 mm	19 mm	3 mm	R
1.2	R	12 mm	17 mm	6 mm	2 mm
Avg (Sample 1)	**15 mm**	**18 mm**	**4.5 mm**	**1.0 mm**	**R**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
2.3	R	6 mm	8 mm	3 mm	5 mm
2.4	R	11 mm	23 mm	7 mm	5 mm
Avg (Sample 2)	**8.5 mm**	**18 mm**	**3.0 mm**	**5.0 mm**	**R**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
3.5	R	19 mm	24 mm	7 mm	11 mm
3.6	R	14 mm	21 mm	4 mm	12 mm
Avg (Sample 3)	**17 mm**	**23 mm**	**7 mm**	**11 mm**	**S**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
4.5	R	8 mm	18 mm	5 mm	12 mm
4.6	R	17 mm	19 mm	2 mm	10 mm
Avg (Sample 4)	**12.5 mm**	**18.5 mm**	**3.5 mm**	**11.0 mm**	**S**
Replicate	**AMP**	**CRO**	**CIP**	**CN**	**C**
5.3	R	7 mm	18 mm	7 mm	6 mm
5.6	R	14 mm	22 mm	4 mm	13 mm
Avg (Sample 5)	**10.5 mm**	**20 mm**	**5.5 mm**	**9.5 mm**	**I**

Legend: AMP (Ampicillin), CRO (Ceftriaxone), CIP (Ciprofloxacin), CN (Gentamicin), and C (Chloramphenicol) were tested. Interpretations follow CLSI 2025: S = Susceptible, I = Intermediate, R = Resistant.

A.5. Antibiotic Susceptibility Results for *Salmonella* spp.: Raw and Averaged Zone Diameters with CLSI Interpretation

APPENDIX A. Cont'd

Antibiotic	Number of Resistant Samples	% Resistance	Interpretation
Ampicillin (AMP)	5/5	(5 ÷ 5) × 100 = 100%	High Resistance
Ceftriaxone (CRO)	2/5	(2 ÷ 5) × 100 = 40%	Moderately Resistant
Ciprofloxacin (CIP)	0/5	(0 ÷ 5) × 100 = 0%	Fully Sensitive
Gentamicin (CN)	5/5	(5 ÷ 5) × 100 = 100%	High Resistance
Chloramphenicol (C)	3/5	(3 ÷ 5) × 100 = 60%	Intermediate Resistance

A.6. Antibiotic Resistance Profile and Computation for *Escherichia coli*

Antibiotic	Number of Resistant Samples	% Resistance	Interpretation
Ampicillin (AMP)	5/5	(5 ÷ 5) × 100 = 100%	High Resistance
Ceftriaxone (CRO)	2/5	(2 ÷ 5) × 100 = 40%	Moderately Resistant
Ciprofloxacin (CIP)	0/5	(0 ÷ 5) × 100 = 0%	Fully Sensitive
Gentamicin (CN)	5/5	(5 ÷ 5) × 100 = 100%	High Resistance
Chloramphenicol (C)	2/5	(2 ÷ 5) × 100 = 40%	Moderately Resistant

A.7. Antibiotic Resistance Profile and Computation for *Salmonella* spp.

APPENDIX B. Informed Consent

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, 2025

Name of Authors: Raven Vhain H. Barong, Trivia Nichole L. Engrasia, Jarto Mae Charisse N. Godinez
College/Department: College of Medical Technology
Research Title: Contamination of Escherichia coli and Salmonella spp. in Rabbitfish (Siganus spp.) Found in Malaubang, Ozamiz City"

Data Gathering Procedure:

a. Type of Research: (Kindly check)

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

b. Respondents/Participants/Subject/Samples to be Collected: Rabbitfish (Siganus spp.)

c. Procedure:

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

d. Place of Implementation/Study Area: Malaubang, Ozamiz City, Misamis Occidental

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

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

e. Date/s of Implementation/Sampling: February first week

Attachments:

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

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

Recommended by:

Jonas T. [signature], MMC, RMT
Thesis/Research Adviser

[signature] Nelza D. Canini PH.D
Thesis/Research Instructor

Approved by:

[signature]
Evangeline H. Veño do, RMT, MATMLS
College Dean

CONTROLLED DOCUMENT

APPENDIX B. 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, Raven Shein M. Barong, to join the experimental research *(activity)* to be held on January to April at Misamis University under the supervision of (Ms. Nelfa Capio)Mr. Jonas T. Hingco *(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.

RIO OBIDO BARONG
Signature over printed name of **Parent/Guardian**

Jan 19, 2025
Date

RAVEN SHEIN M. BARONG
Signature over printed name of **Student**

Jan 19, 2025
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 14 JAN 2025 day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 99
Page No. 21
Book No. XXVII
Series of 2025

ATTY. DANIEL C. LAO
Notary Public
For the City of Ozamiz and the Province of Misamis Occidental
Until December 31, 2025
Notarial Commission No. 2023-12
PTR No. 5597293 01/07/2025-Ozamiz City
IBP No. 02373 (Lifetime)
TIN-135-323-084
Roll No. 29112

(For DSAS Personnel only)

Attested by:

NELPA N. CAPIO, LPT, MAEd, JD
Director, Department of Student Affairs & Services

APPENDIX B. 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, Trisia Nichole L. Engracia, to join the Experimental research *(activity)* to be held on January to April at Misamis University under the supervision of Mr. Jonas T. Hingco *(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.

Signature over printed name of **Parent/Guardian** — Date: 1-14-25

Signature over printed name of **Student** — Date: 1-14-25

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 14 JAN 2025 day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 01
Page No. 01
Book No. VIII
Series of 2025

ATTY. DANIEL C. LAO
Notary Public
Notary Public For the City of Ozamiz and the Province of Misamis Occidental
Until December 31, 2025
Notarial Commission No. 2023-12
PTR No. 5597259 LO 01/07/2025-Ozamiz City
IBP No. 02373 (Lifetime)
TIN-135-323-064
Roll No. 29412

(For DSAS Personnel only)

Attested by:

NELPA N. CAPIO, LPT, MAEd, JD
Director, Department of Student Affairs & Services

APPENDIX B. 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, Tasha Mae Charisse N. Godinez, to join the experimental research *(activity)* to be held on January to April at Misamis University under the supervision of Mr. Jonas T. Hingco *(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.

Signature over printed name of **Parent/Guardian** — 01/14/25 Date

Signature over printed name of **Student** — 01/14/25 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 14 JAN 2025 at OZAMIZ CITY Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 20
Page No. 04
Book No. VIII
Series of 2025

ATTY. DANIEL C. LAO
Notary Public
For the City of Ozamiz and the Province of Misamis Occidental
Until December 31, 2025
Notarial Commission No. 2023-12
PTR No. 5597268-01/07/2025-Ozamiz City
IBP No. 02373 (LIFE Member)
TIN-135-323-064
Roll No. 09112

(For DSAS Personnel only)

Attested by:

NELPA M. CAPIO, LPT, MAEd, JD
Director, Department of Student Affairs & Services

APPENDIX C. Approved Letters

Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY

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

January 22, 2025
Nelfa D. Canini, Ph.D.
Head of Natural Sciences
Misamis University
5R2R+386, H.T. Feliciano St, Ozamiz City, 7200 Misamis Occidental

Subject: Request for Permission to use the Natural Science Laboratory and Laboratory Equipment for Research Purposes

Dear Ma'am Canini,

We, the student researchers from the College of Medical Technology at Misamis University, writing to request your permission to borrow laboratory equipment essential and use the Natural Science laboratory for our research study entitled **"Contamination of *Escherichia coli* and *Salmonella spp.* in Rabbitfish *(Siganus spp.)* Found in Malaubang, Ozamiz City."**

The equipment will be used to ensure the proper execution of our experiments and data collection. We assure you that all items will be handled responsibly, following laboratory protocols and safety guidelines, and will be returned to their original condition. Should you need further details about the research or the equipment, we are happy to provide additional information.

Thank you for considering this request. We look forward to your favorable response and continued support. Please feel free to contact us at godinezmae2003@gmail.com or phone number 09206762875/09082575538 for any clarifications.

Yours respectfully,

RAVEN SHEIN M. BARONG, BS MEDTECH
Student Researcher

TRISIA NICHOLE L. ENGRACIA, BS MEDTECH
Student Researcher

TASHA MAE CHARISSE N. GODINEZ, BS MEDTECH
Student Researcher

APPENDIX C. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

Noted by:

JONAS T. HINGCO, MSc.Bio
Research Adviser—College of Arts and Sciences

Approved by:

NELFA D CANINI, Ph.D.
Chairman of the Department of Natural Sciences

APPENDIX C. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

January 22, 2025

Mrs. Evangeline M. Señedo
Dean of the College of Medical Technology
Misamis University
SR2R+386, H.T. Feliciano St, Ozamiz City, 7200 Misamis Occidental

Subject: Request for Permission to use the Microbiology Laboratory and Laboratory Equipment for Research

Dear Dean Señedo,

We, the student researchers from the College of Medical Technology at Misamis University, writing to request your permission to borrow laboratory equipment essential and use the microbiology laboratory for our research study entitled **"Contamination of *Escherichia coli* and *Salmonella spp.* in Rabbitfish *(Siganus spp.)* Found in Malaubang, Ozamiz City,"**

The equipment will be used to ensure the proper execution of our experiments and data collection. We assure you that all items will be handled responsibly, following laboratory protocols and safety guidelines, and will be returned to their original condition. Should you need further details about the research or the equipment, we are happy to provide additional information.

Thank you for considering this request. We look forward to your favorable response and continued support. Please feel free to contact us at godinezmae2003@gmail.com or phone number 09206762875/09082575538 for any clarifications.

Yours respectfully,

RAVEN SHEIN M. BARONG, BS MEDTECH
Student Researcher

TRISIA NICHOLE L. ENCRACIA, BS MEDTECH
Student Researcher

TASHA MAE CHARISSE N. GODINEZ, BS MEDTECH
Student Researcher

APPENDIX C. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

Noted by:

JONAS T. HINGCO, MSc.Bio
Research Adviser—College of Arts and Sciences

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean of the College of Medical Tecgnology

APPENDIX C. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

January 22, 2025
Hon. Cris Maghanoy
Barangay Captain
Barangay Malaubang
Ozamis City, Misamis Occidental

Subject: Request for Permission to Conduct Research in Barangay Malaubang

Dear Hon. Cris Maghanoy,

We, the student researchers from the College of Medical Technology at Misamis University are writing to formally request permission to conduct our research study entitled, **"Contamination of *Escherichia coli* and *Salmonella spp.* in Rabbitfish *(Siganus spp.)* Found in Malaubang, Ozamiz City,"** within your barangay. Our study aims to assess the microbiological contamination of rabbitfish *(Siganus spp.)* and its aquatic environment in Malaubang, Ozamiz City, with a focus on detecting pathogenic bacteria and evaluating their antibiotic resistance profiles. As part of our methodology, we intend to collect fish sample and water samples from your barangay with the assistance of local fishermen. We respectfully seek your approval to conduct our research in the area and engage with the fishermen for sample collection. Their knowledge and experience in the local fishing environment will be invaluable in ensuring the success of our study.

We kindly seek your approval and support for the following:

1. Conducting our research activities within Barangay Malaubang.
2. Collaborating with local fishermen to assist in the collection of fish sample and water samples from Malaubang.

We believe that the results of this study could provide valuable insights into addressing potential health risks associated with fish consumption and water quality in the community. We are more than willing to discuss our research objectives and procedures in detail at your convenience. We appreciate your time and consideration and look forward to your positive response.

Thank you for considering our request. We look forward to your positive response and are hopeful for your support in this endeavor. Should you have any questions or require further information,

APPENDIX C. Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

please do not hesitate to contact us at godinezmac2003@gmail.com or phone number 09206762875/09082575538

Yours respectfully,

RAVEN SHEIN M. BARONG, BS MEDTECH
Student Researcher

TRISIA NICHOLE L. ENGRACIA, BS MEDTECH
Student Researcher

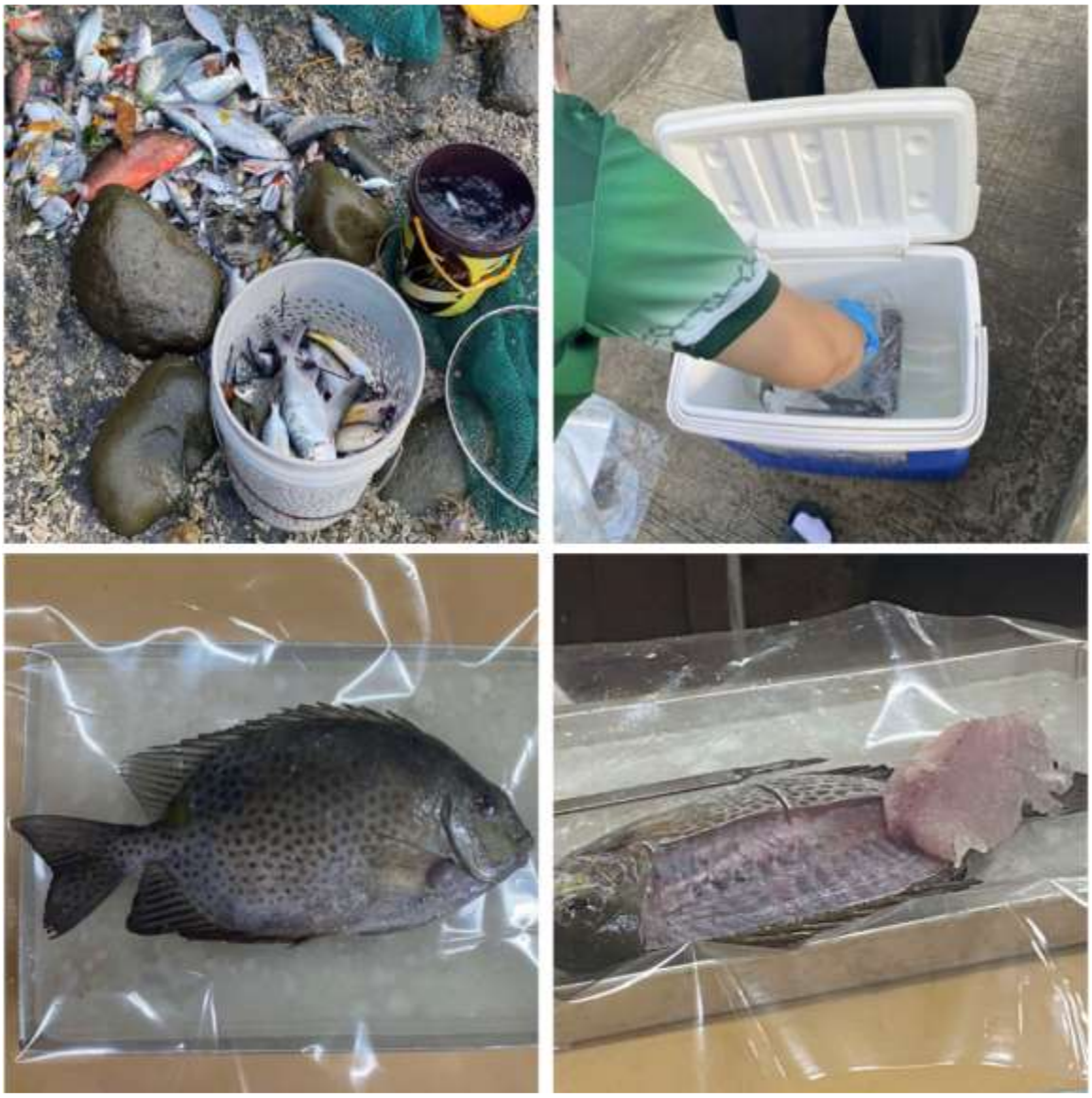
TASHA MAE CHARISSE N. GODINEZ, BS MEDTECH
Student Researcher

Noted by:

JONAS T. HINGCO, MSc.Bio
Research Adviser—College of Arts and Sciences

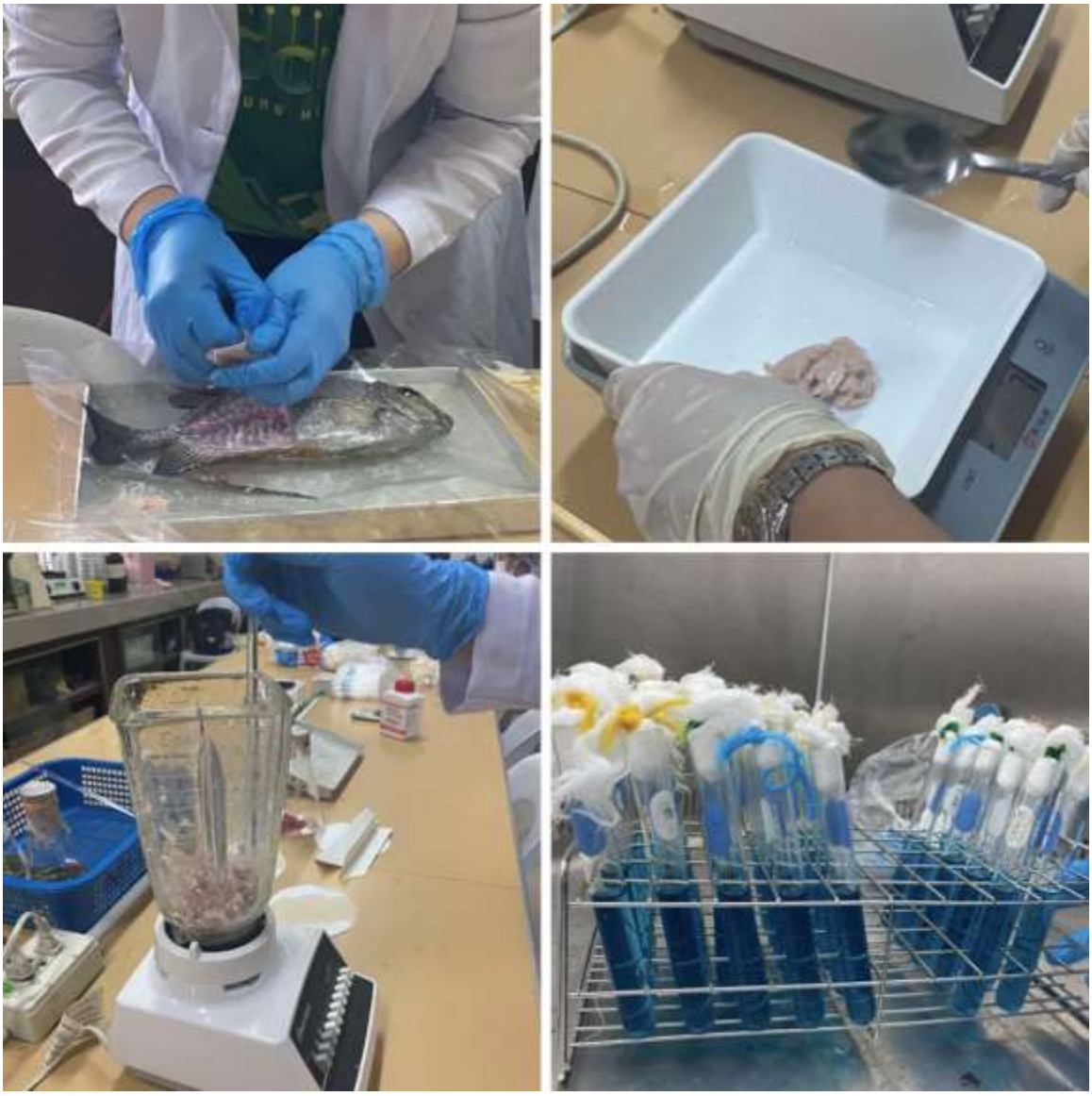
EVANGELINE M. SENEDO, RMT, MATMRS
Dean of the College of Medical Technology

APPENDIX D. Documentations



D.1. Sample Collection, Handling, and Preparation

APPENDIX D. Cont'd

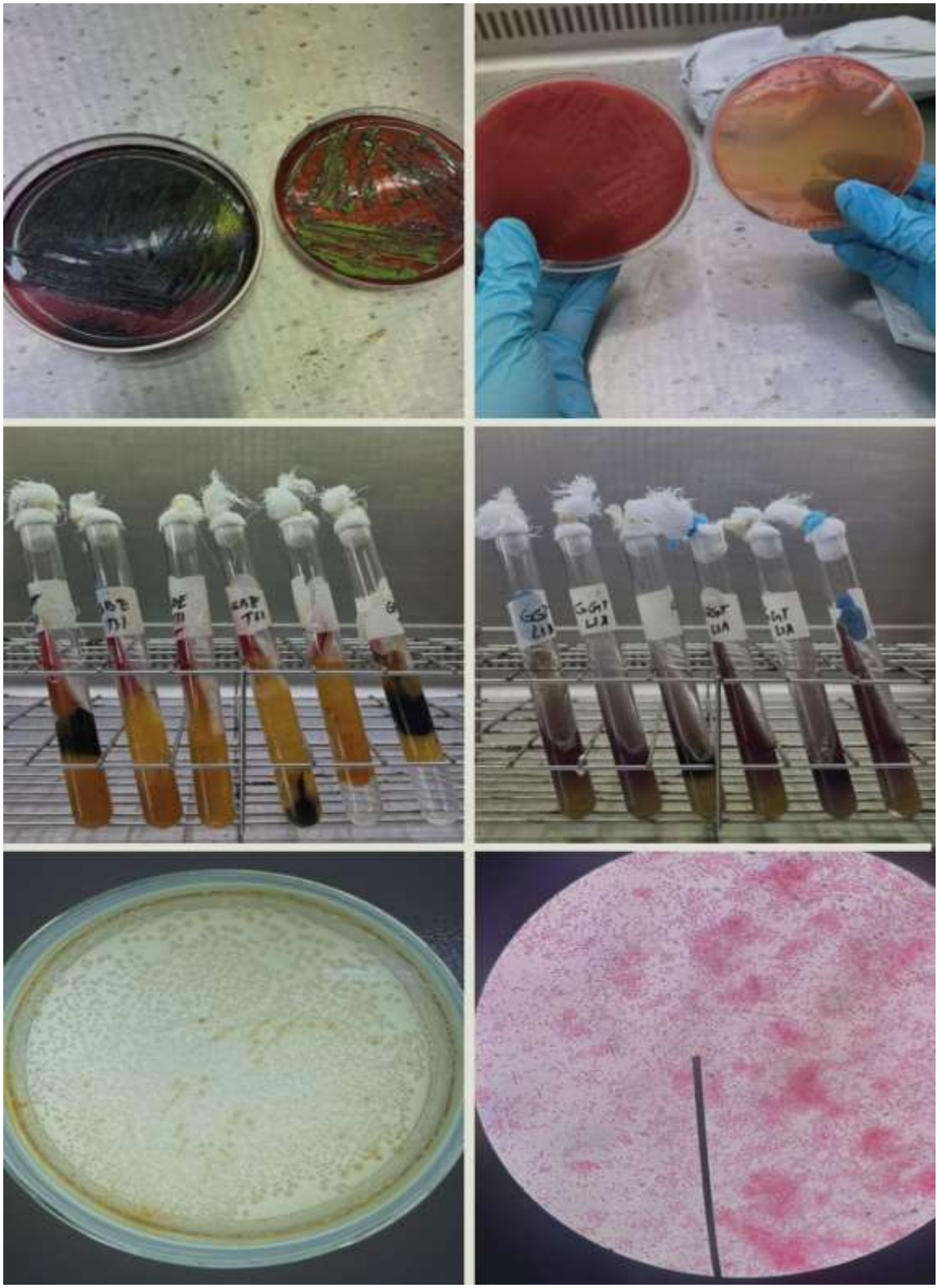


D.2. Microbial Analysis of Fish Samples

APPENDIX D. Cont'd

D.3. Microbial Analysis - Continuation

APPENDIX D. Cont'd



D.4. Biochemical Tests Overview

APPENDIX D. Cont'd

D.5. Antibiotic Susceptibility Testing Overview

APPENDIX D. Cont'd

D.6. *Escherichia coli* Control

D.7. *Salmonella* spp. Control

APPENDIX D. Cont'd

Replicate	Colonies Counted	Dilution	Volume Plated
Number 1.0	371	7^{th}	0.1 ml
Number 1.1	127	7^{th}	0.1 ml
Number 2.1	326	7^{th}	0.1 ml
Number 2.2	395	7^{th}	0.1 ml
Number 3.0	319	7^{th}	0.1 ml
Number 4.2.1	421	7^{th}	0.1 ml
Number 4.2.2	254	6^{th}	0.1 ml
Number 4.2.3	291	6^{th}	0.1 ml
Number 4.2.4	211	6^{th}	0.1 ml
Number 4.2.5	170	6^{th}	0.1 ml
Number 5.0	328	6^{th}	0.1 ml
Number 5.1	263	6^{th}	0.1 ml

D.8. Raw Data of *Escherichia coli* (From EMB Agar)

Replicate	Colonies Counted	Dilution	Volume Plated
Number 1.0	157	7^{th}	0.1 ml
Number 1.1	143	7^{th}	0.1 ml
Number 1.2	220	7^{th}	0.1 ml
Number 2.1	178	7^{th}	0.1 ml
Number 2.2	902	7^{th}	0.1 ml
Number 2.3	222	7^{th}	0.1 ml
Number 3.0	309	7^{th}	0.1 ml
Number 4.0	335	7^{th}	0.1 ml
Number 4.2.1	387	6^{th}	0.1 ml
Number 4.2.2	706	6^{th}	0.1 ml
Number 4.2.3	538	6^{th}	0.1 ml
Number 4.2.4	324	6^{th}	0.1 ml
Number 5.0	321	6^{th}	0.1 ml
Number 5.1	208	6^{th}	0.1 ml
Number 5.2	516	6^{th}	0.1 ml
Number 5.3	318	6^{th}	0.1 ml

D.9. Raw Data of *Escherichia coli* (From PCA Agar) – Confirmatory Test

APPENDIX D. Cont'd

Replicate	Colonies Counted	Dilution	Volume Plated
Number 1.3	108	7^{th}	0.1 ml
Number 2.1	264	7^{th}	0.1 ml
Number 2.5	284	7^{th}	0.1 ml
Number 3.2	136	7^{th}	0.1 ml
Number 3.3	306	7^{th}	0.1 ml
Number 3.4	411	7^{th}	0.1 ml
Number 4.2	17	7^{th}	0.1 ml
Number 4.3	Colony cannot be counted.	7^{th}	0.1 ml
Number 4.3	Colony cannot be counted.	7^{th}	0.1 ml
Number 4.4	38	7^{th}	0.1 ml
Number 4.5	426	6^{th}	0.1 ml
Number 5.1	85	6^{th}	0.1 ml
Number 5.1.0	51	6^{th}	0.1 ml
Number 5.2	181	6^{th}	0.1 ml
Number 5.2.1	79	6^{th}	0.1 ml
Number 5.3.0	236	6^{th}	0.1 ml
Number 5.4	47	6^{th}	0.1 ml
Number 5.4.1	73	6^{th}	0.1 ml
Number 5.5	33	6^{th}	0.1 ml
Number 5.5.0	105	6^{th}	0.1 ml

D.10. Raw Data of *Salmonella* spp. (From SSA Agar)

APPENDIX D. Cont'd

Replicate	AMP	CRO	CIP	CN	C
Number 1.1	R	1.2 cm	2.6 cm	0.9 cm	0.6 cm
Number 1.2	R	1.0 cm	2.6 cm	0.5 cm	0.3 cm
Number 2.3	R	2.0 cm	2.1 cm	0.2 cm	1.1 cm
Number 2.4	R	1.6 cm	1.8 cm	0.3 cm	0.4 cm
Number 3.5	R	1.9 cm	2.4 cm	0.7 cm	1.1 cm
Number 3.6	R	1.1 cm	2.4 cm	0.7 cm	0.9 cm
Number 4.1	R	1.7 cm	1.7 cm	0.2 cm	0.3 cm
Number 4.2	R	0.9 cm	1.1 cm	0.6 cm	0.4 cm
Number 5.3	0.2 cm	2.3 cm	2.1 cm	0.5 cm	1.9 cm
Number 5.4	R	1.2 cm	2.0 cm	0.2 cm	0.4 cm

Note: Antibiotics and their corresponding abbreviations used in this study are as follows: Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamicin (CN), and Chloramphenicol (C). The designation "R" indicates resistance in the susceptibility results.

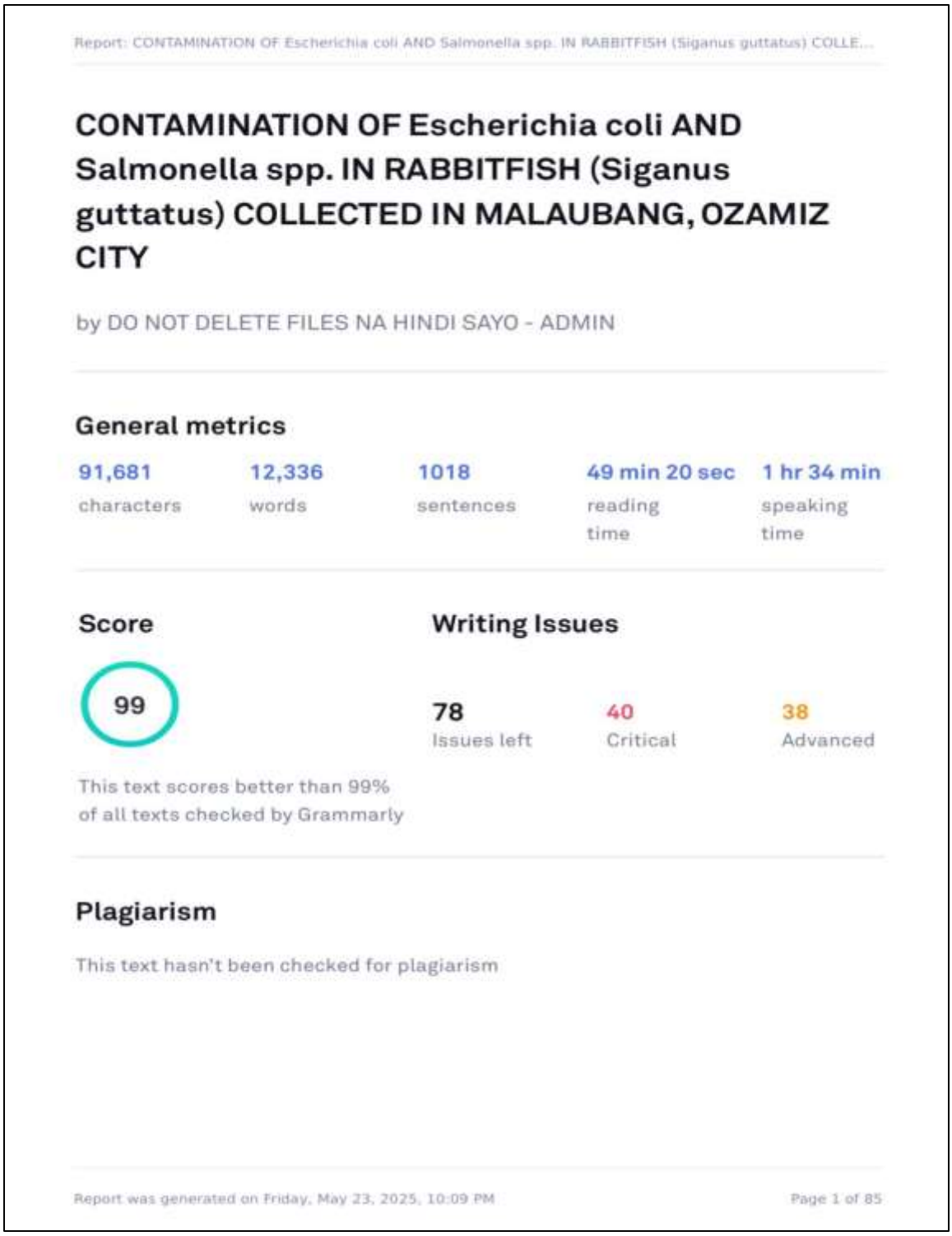
D.11. Raw Data on the Measurement of the Zone of Inhibition for *Escherichia coli*

Replicate	AMP	CRO	CIP	CN	C
Number 1.1	R	1.8 cm	1.9 cm	0.3 cm	R
Number 1.2	R	1.2 cm	1.7 cm	0.6 cm	0.2 cm
Number 2.3	R	0.6 cm	1.8 cm	0.3 cm	0.5 cm
Number 2.4	R	1.1 cm	2.3 cm	0.7 cm	0.5 cm
Number 3.5	R	1.9 cm	2.4 cm	0.7 cm	1.1 cm
Number 3.6	R	1.4 cm	2.1 cm	0.4 cm	1.2 cm
Number 4.5	R	0.8 cm	1.8 cm	0.5 cm	1.2 cm
Number 4.6	R	1.7 cm	1.9 cm	0.2 cm	1.0 cm
Number 5.3	R	0.7 cm	1.8 cm	0.7cm	0.6 cm
Number 5.6	R	1.4 cm	2.2 cm	0.4 cm	1.3 cm

Note: Antibiotics and their corresponding abbreviations used in this study are as follows: Ampicillin (AMP), Ceftriaxone (CRO), Ciprofloxacin (CIP), Gentamicin (CN), and Chloramphenicol (C). The designation "R" indicates resistance in the susceptibility result.

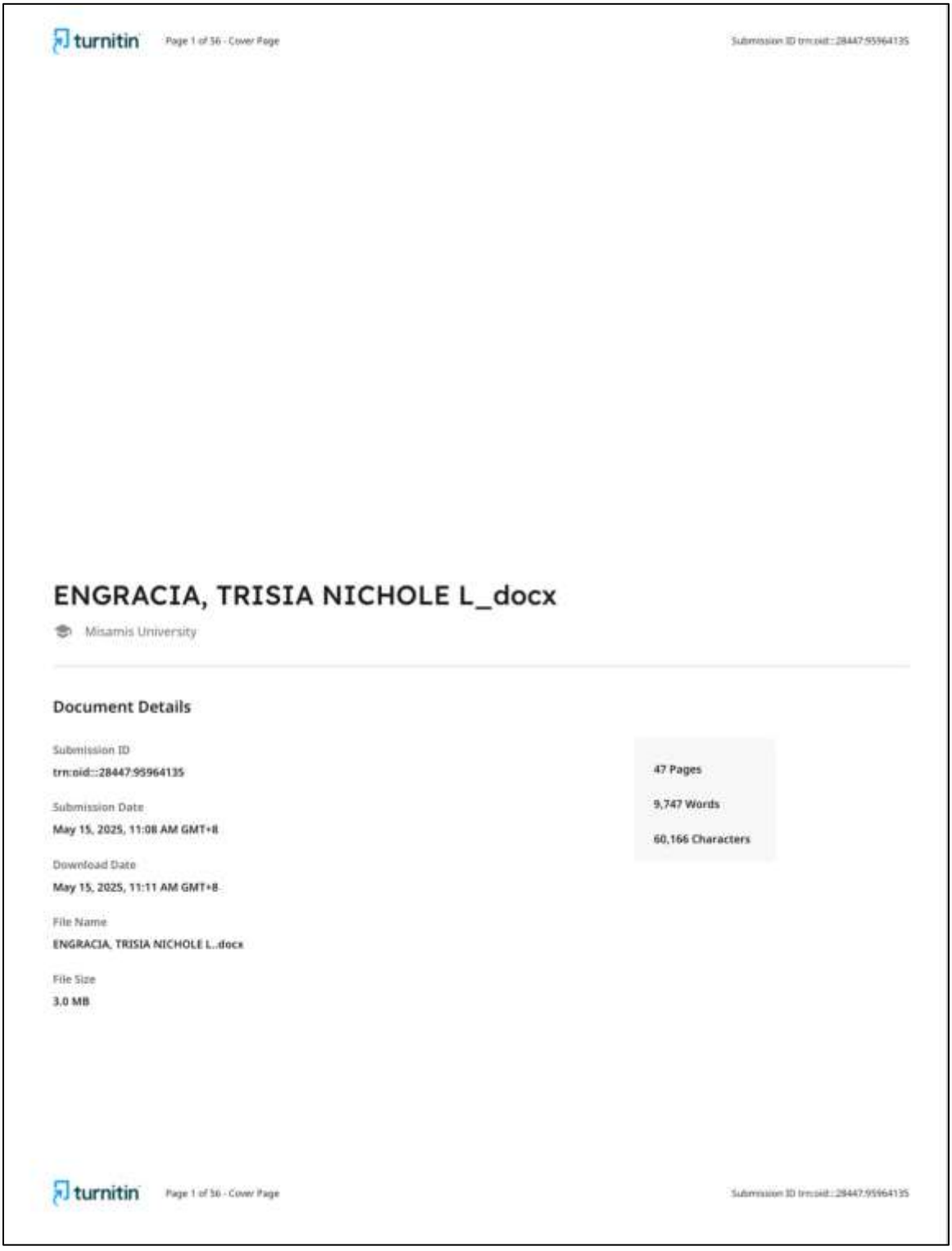
D.12. Raw Data on the Measurement of the Zone of Inhibition for *Salmonella* spp.

APPENDIX E. Grammarly Reports and Turnitin Results



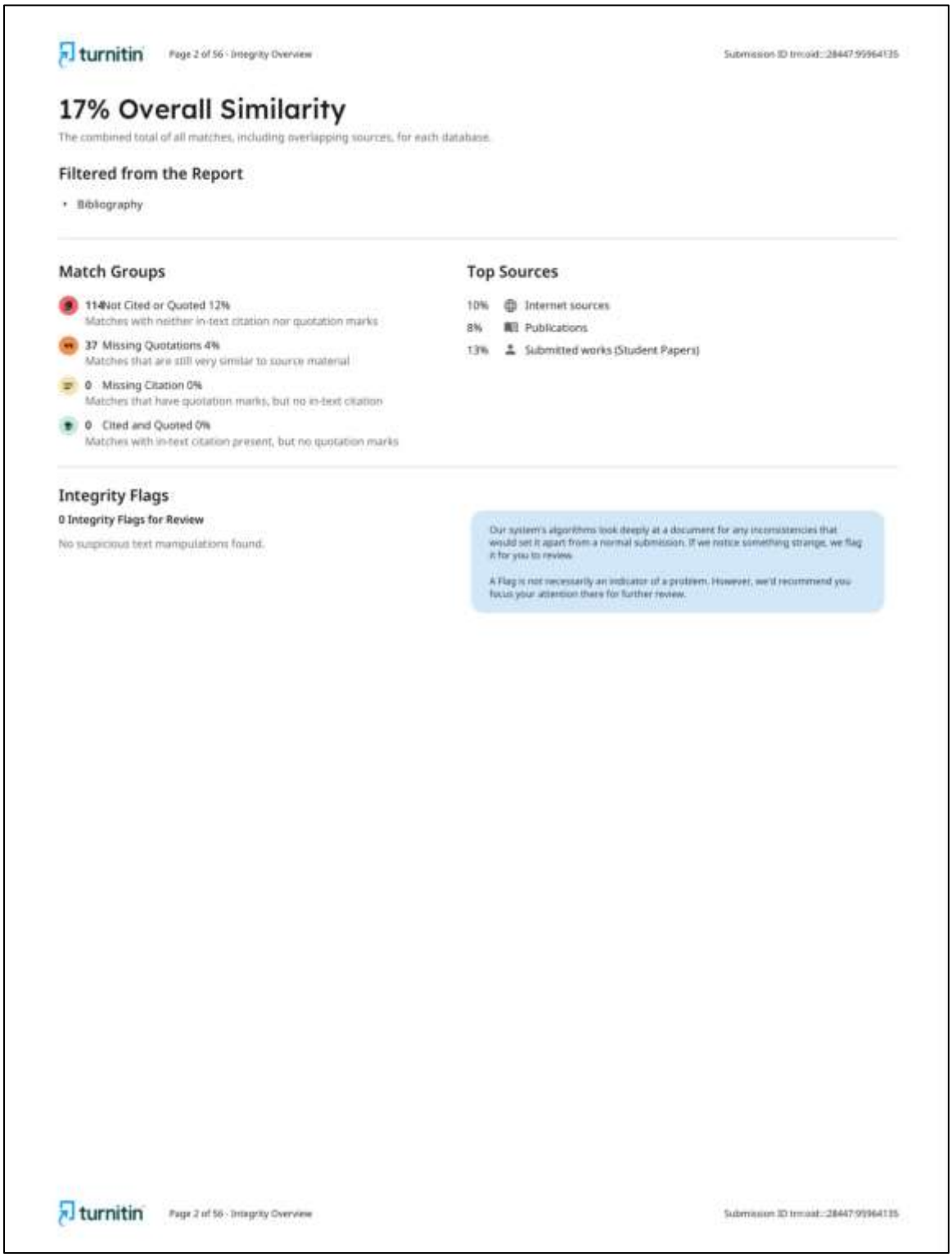
E.1. Grammarly Report

APPENDIX E. Cont'd



E.2. Turnitin Result

APPENDIX E. Cont'd



E.3. Turnitin Result

CURRICULUM VITAE

PERSONAL INFORMATION

Name	:	Raven Shein M. Barong
Age	:	20
Birthdate	:	July 19, 2004
Civil Status	:	Single
Nationality	:	Filipino
Home Address	:	Purok 3, Sangay Daku, Ozamiz City, Misamis Occidental
Current Address	:	Purok 3, Sangay Daku, Ozamiz City, Misamis Occidental
Contact Number	:	09381475346
E-mail Address	:	nevarsMbarong@gmail.com

EDUCATIONAL BACKGROUND

Tertiary	:	Bachelor of Science in Medical Technology Misamis University Ozamiz City, Misamis Occidental 2022 – Present
Secondary	:	Northwestern Mindanao State College Labuyo, Tangub City, Misamis Occidental 2020 – 2022
	:	San Isidro High School Lower Lodiong, Tambulig, Zamboanga Del Sur 2016 – 2020
Primary	:	Paterno Gallego Elementary School Linconan, Bonifacio, Misamis Occidental 2011 – 2016

PERSONAL INFORMATION

Name	:	Trisia Nichole L. Engracia
Age	:	21
Birthdate	:	January 25, 2004
Civil Status	:	Single
Nationality	:	Filipino
Home Address	:	RS TAN Village, Maningcol, Ozamiz City, Misamis Occidental
Current Address	:	RS TAN Village, Maningcol, Ozamiz City, Misamis Occidental
Contact Number	:	09082575538
E-mail Address	:	trisianggg@gmail.com

EDUCATIONAL BACKGROUND

Tertiary	:	Bachelor of Science in Medical Technology Misamis University Ozamiz City, Misamis Occidental 2022 – Present
Secondary	:	Misamis University Ozamiz City, Misamis Occidental 2016 – 2022
Primary	:	Misamis University Ozamiz City, Misamis Occidental 2011 – 2016

PERSONAL INFORMATION

Name	:	Tasha Mae Charisse N. Godinez
Age	:	21
Birthdate	:	October 01, 2003
Civil Status	:	Single
Nationality	:	Filipino
Home Address	:	Purok 1, Gango, Ozamiz City, Misamis Occidental
Current Address	:	Purok 1, Gango, Ozamiz City, Misamis Occidental
Contact Number	:	09206762875
E-mail Address	:	godinezmae2003@gmail.com

EDUCATIONAL BACKGROUND

Tertiary	:	Bachelor of Science in Medical Technology
		Misamis University
		Ozamiz City, Misamis Occidental
		2022 – Present
Secondary	:	Misamis University
		Ozamiz City, Misamis Occidental
		2018 – 2022
	:	Ozamiz City School of Arts and Trades
		Maningcol, Ozamiz City, Misamis Occidental
		2016 – 2018
Primary	:	Gango Elementary School
		Purok 4, Gango, Ozamiz City, Misamis Occidental
		2011 – 2016