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Research Article

Antimicrobial Resistance and Molecular Identification of *Vibrio* spp. in Shellfish at Major Public Wet Markets in Bukidnon, Philippines.

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A B S T R A C T.

Vibrio species are particularly pathogenic and pose significant public health risks when shellfish become contaminated from aquatic ecosystem. This study assessed the occurrence, molecular identification, and antimicrobial resistance of *Vibrio* species isolated from mud clams, mud crabs, and white leg shrimp sold in three public markets in Bukidnon, Philippines. A high occurrence (55%) of these bacteria was observed across all locations. Molecular identification of fifteen bacterial isolates was performed using 16s rRNA gene sequencing followed by BLAST analysis against public databases. The results revealed the closes similarity to *V. alginolyticus* (5 isolates), *Vibrio* sp. (3 isolates), *V. furnissii* (2 isolates), and *V. parahaemolyticus* (5 isolates). Maramag public market has sample occurrence of 44.4% (4), Malaybalay 55.5% (5), and Valencia 66.6% (6). Accorss samples, the mud clam exhibited the highest occurrence rate of 88.8%, followed by shrimp at 33.3%, and mud crab at 30%. Antimicrobial susceptibility testing through disk diffusion method, following standardized inoculation, disc placement, incubation, and zone reading procedures of fifteen isolates, among them, 100% were resistance to ampicillin and 60% to cefotaxime, with 66% exhibiting multidrug resistance. The multidrug-resistant phenotype involved resistance to multiple antibiotic classes, including beta-lactams, macrolides, cephalosporin, and fluroquinolones. These findings point the presence of multi-drug resistant *Vibrio* spp., suggesting potential food safety risks and underscoring the need for ongoing monitoring and management to mitigate *Vibrio* related health threats in the region.The abstract should have a brief and factual presentation of the research purpose, major results, and main conclusion and should be presented separately. Citing references in the abstract, as well as mentioning non-standard or uncommon abbreviations must be avoided. It is imperative that are not supported by the main text or exaggerating the primary conclusions.

Keywords: Vibrio, Antimicrobial Resistance, Public Market

INTRODUCTION

Vibrio species are associated with severe vibriosis in both marine aquaculture and humans, with a history of seafood cosumption being a major risk factor for infection. It threatens human health, causing considerable illness, gastrointestinal disease, sepsis, and mortality worldwide (El-Zamkan et al., 2023; Osunla & Okoh, 2017). This species also infects economically important aquatic animals such as fish, shrimp, and shellfish (Cai et al., 2024; George et al., 2005; Norfolk et al., 2023). It causes acute hepatopancreatic necrosis syndrome (AHPNS), larval and juvenile mortality in seafood (Gómez-León et al., 2005; Yin et al., 2022).

Vibrio harbor key virulence factors. The ToxR regulon is a hierarchical regulatory system that controls the expression of significant virulence genes, cholera toxin (CT), and toxin-coregulated pilus (TCP) (J. Lee, 2023). The ctx genes (ctxA and ctxB) encode cholera toxin (CTX), a significant virulence factor linked to V. cholerae O1 and O139 strains (Potasman et al., 2002). Toxin-coregulated pilus (TcpP), a regulatory protein, positively modulates the expression of virulence genes in V. cholerae, including CTX and colonization factors like TCP (Häse & Mekalanos, 1998). TcpP is vital for V. cholerae infection by enhancing the expression of critical virulence genes (D. Lee et al., 2023). Virulence factors are crucial for pathogenic bacteria to evade host immune responses (Beceiro et al., 2013).

Antibiotics remain the most common treatment for vibriosis in aquaculture animals and in human infections. Effective antibiotics against Vibrio infections include tetracycline, quinolones, trimethoprim, potentiated sulfonamides, oxolinic acid, and sarafloxacin (Laganà et al., 2011; Yano et al., 2014). However, large quantities of antibiotics are administered in aquaculture, which contributes significantly to the development of antimicrobial resistance (AMR) (Nikaido, 2009).

Additionally, resistance determinants often reside in plasmids that can be transferred to other bacterial species through horizontal gene transfer mechanisms (Tao et al., 2022). Thus, AMR could spread across bacterial populations. Reports on Vibrio alginolyticus have been resistant to cephalotin, amikacin, cephotaxime, pefloxacin, ampicillin, and vancomycin (Kang et al., 2016; Soyer-Gobillard, 2017).

The European Food Safety Authority (2024) emphasizes the importance of identifying *Vibrio* species in seafood, noting that climate change-driven increases in sea surface temperatures promote their proliferation. Consequently, these bacteria post significant health risks, and warrant accurate monitoring and control in seafood production. Rising surface water temperatures resulting from global warming are driving worldwide increase in *Vibrio* outbreaks linked to contaminated seafoood (Onohuean et al., 2022; Vezzulli et al., 2015).

Public markets play a significant role in the dissemination and potential transmission of *Vibrio* species, as they serve as central hubs for the sale and handling of shellfish and other fresh produce, often under conditions or cross-contamination that can facilitate the spread of pathogens (Teh et al., 2020). Contamination of seafood products can occur during animal rearing, preparation, transport, and through food handlers working in unsanitary environments (WHO, 2019). Several studies in the Philippines have revealed *Vibrio* contamination in green mussels (Perna viridis) from Bacoor Bay, Cavite (Tabo et al., 2015). *Vibrio* spp. in tilapia from Pampanga (Aquino, 2022). Vibrio harveyi-like bacteria linked to fin rot in farmed milkfish fingerlings (Estante-Superio et al., 2021). A recently study also detected *Vibio* spp. in retail shrimp from General Santos City and Sarangani Province (Danao & Rallos, 2025).

However, there is limited data on the occurrence and contamination levels of *Vibrio* in seafood sold at wet markets across Mindanao, the country's second-largest island. Bukidnon, a landlocked province within Mindanao, relies heavily on wet markets for its seafood supply, yet there remain no specific studies addressing *Vibrio* contamination in these products. National-level monitoring of *Vibrio* prevalence in the Philippines' seafood is currently insufficient, highlighting the urgent need for comprehensive surveillance programs to

evaluate and mitigate food safety and public health risks. Sustained monitoring of *Vibrio* spp. and sustain monitoring of AMR *Vibrio* spp. in seawater, shellfish, and market environments is essential for preventing future outbreaks and safeguarding both consumer and ecosystem health.

METHODOLOGY

2.2 Collection of Shellfish Samples

The samples were aseptically purchased from the major public markets of Malaybalay City, Valencia City, and Maramag, Bukidnon (Figure 1). The shellfish with three samples from each market are mud clams (*Geloina striata Linne*), mud crabs (*Scylla* spp.), and white leg shrimp (*Litopenaeus vannamei*). The composite samples consist of 250 g of each seafood type. These samples were randomly collected aseptically from each stall, with three replicates of each sample per market, and placed in sterile plastic bags. After purchase, samples were transported to the Tuklas Lunas Development Center laboratory, Central Mindanao University on ice packs in cooler boxes within two hours of collection.

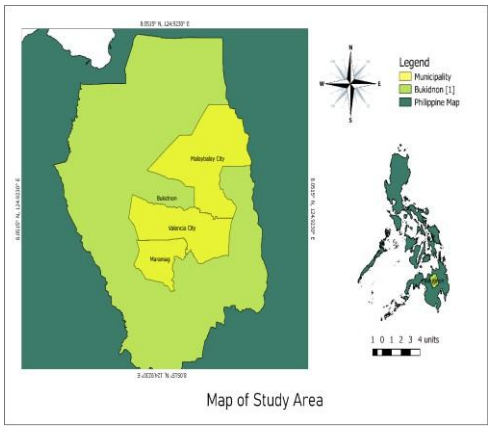

Figure 1. Location of sampling sites/public markets in the Province of Bukidnon.

2.3 Sample Processing, Isolation, and Purification of Isolates

Sample preparation was conducted according to the Bacteriological Analytical Manual guidelines (FDA, 2022). Each 25 g sample was added to 225 mL of alkaline peptone water (APW) (Condalab, Madrid, Spain) and homogenized using an immersion blender. The APW samples were transferred into Erlenmeyer flasks and incubated at 37°C for 24 hours.

The enrich culture in APW was serially diluted up to 10^5 in test tubes containing 9 mL saline solution (Himedia, India). Each diluent (100 µL) was spread onto Thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates (Himedia, India). After 24 hours of incubation at 37°C, the presumptive *Vibrio* colonies measuring 2–4 mm in diameter, yellow with opaque centers and translucent edges, were identified. These colonies were then sub-cultured on TCBS agar plates using the streak plate method to obtain pure isolates.

2.4 Antimicrobial Susceptibility Testing

Vibrio isolates were subjected to antimicrobial susceptibility testing. The antibiogram of each of the *Vibrio* isolates was determined through the standard disk diffusion assay (CLSI, 2012). A total of eight (8) antibacterial agents (Bioanalyse, Turkey) namely: ampicillin (AP) 10 µg, azithromycin (ATH) 15 µg, cefotaxime (CTX) 30 µg, chloramphenicol (C) 30 µg, sulphonamides 250 µg, tetracycline (TET) 30 µg, trimethoprim-sulfamethoxazole (TM-SMX) 5 µg, meropenem (MEM) 10 µg, and ciprofloxacin (CIP) 5 µg were used. These antibiotics were selected based on their frequent use in clinical practices and according to the Clinical and Laboratory Standards Institute (CLSI) M45 and M100 guidelines for *Vibrio*.

A direct colony suspension was prepared by suspending bacterial colonies in a 0.85% NaCl solution and adjusting the turbidity to a 0.5 McFarland standard. Using a sterile cotton swab, the standardized inoculum was evenly swabbed onto a Mueller-Hinton agar (MHA) plate (Himedia, India). After the placement of the antibiotic discs, the placement was allowed to dry for 5–10 minutes before incubation, permitting proper disc contract with the agar; antibiotic diffusion occurred during incubation. The Petri dish used was 90 mm in diameter. The distance between the antibiotic discs was 20-24 mm edge to edge, and the plates were incubated at 35°C for 16–20 hours. The zone of inhibition for each antibiotic was measured in millimeters with a ruler. The established zone of inhibition (ZOI) breakpoints for each antibiotic against *Vibrio* spp., set by the Clinical and Laboratory Standards Institute (CLSI) M45 and M100 guidelines, were used to classify the isolates as susceptible, intermediate, or resistant.

2.5 Determination of Multiple Antibiotic Resistance Index (MARI)

The MARI was calculated and interpreted for each isolate, sample type, and sampling area as recommended.

The isolates with a MARI of ≥ 0.2 indicate that they originated from an environment with high antibiotic exposure. The MARI was calculated using a formula adopted from Abioye et al. (2023) as shown below:

MARI from an isolate = a/b

Where a = number of antibiotics an isolate exhibited resistance against, and b = the total number of antibiotics tested against the isolate.

2.6 Sequencing and Data Analysis

The *Vibrio* isolates in glycerol stock were sent to Macrogen, Incorporated, located at 908 World Meridian Venture Center, No.60-24 Gasan Dong, Geumchun-gu, Seoul, South Korea. for sequencing. MEGA 5.05 software was used to assemble the sequences. The isolates were sequenced for 16S rRNA, F(5′-AGAGTTTGATCCTGGCTCAG-3′), R(5′-GGTTACCTTGTTACGACTT-3′) for identification. Subsequently, these sequences were compared to entries in GenBank using the BLAST search engine and submitted to the NCBI (http://www.ncbi.nlm.gov/blast/).

RESULTS AND DISCUSSION

3.1 Occurrence of *Vibrio* Isolates

Molecular identification of twenty-seven bacterial isolates was performed using *16S rRNA* gene sequencing followed by BLAST analysis against public databases. The results revealed the closest similarity to *Vibrio alginolyticus* (5/27), *Vibrio sp*. (3/27), *Vibrio furnissii* (2/27), and *Vibrio parahaemolyticus* (5/27). The occurrence of *Vibrio* species from samples is parallel to the study of Alavandi (2006), where *Vibrio* species have also been recovered from seafood sources such as green mussels, indicating the broad distribution of these bacteria in marine food products. Additionally, *Vibrio* species, including *V. cholerae*, *V. harveyi*, *Vibrio furnissii*, and *V. fluvialis*, have been from blue crabs (*Callinectes sapidus*), an important commercially harvested crustacean by Omogbai (2016). Gómez-León (2005) isolated *V. alginolyticus* and *V. splendidus* biovar II from moribund carpet shell clam larvae, implicating these species in aquaculture disease outbreaks. Furthermore, occurrences of *Vibrio* species have been documented in cultured white leg shrimp (*Litopenaeus vannamei*), which is a major species in shrimp aquaculture (Danao & Rallos, 2025).

The use of Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar was crucial in isolating *Vibrio* species. Glackin (2024) demonstrated the effectiveness of combining TCBS agar with CHROMagar for isolating *Vibrio* spp. from environmental samples. Their method involved directly plating water and sediment onto TCBS agar, incubating the plates, and counting the colonies, followed by molecular confirmation. Authoritative guidelines from the U.S. Centers for Disease Control and Prevention (CDC, 2024) recommend TCBS agar as the preferred selective medium for isolating *V. cholerae* from both fecal and environmental samples. When combined with enrichment in alkaline peptone water, TCBS agar enhances recovery rates and facilitates reliable identification through its characteristic colony appearance.

The reliability of TCBS agar is further supported by the earlier work of Uchiyama (2000), who successfully isolated multiple *Vibrio* species—including *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*—from various aquatic environments such as rivers, estuaries, and seawater. This confirms the medium's usefulness as a standard in aquatic microbiology for identifying and isolating *Vibrio* spp.. Tagliavia (2019) further support the essential role of TCBS agar in *Vibrio* isolation but recommend modifications to expand the recovery range of marine *Vibrio* species. These adjustments could improve the medium's selectivity and sensitivity, highlighting ongoing efforts to refine microbiological detection methods.

Vibrio species are important foodborne pathogens capable of causing gastrointestinal illness and, in some cases, severe extraintestinal infections. Although many *Vibrio* isolates remain susceptible to commonly used antibiotics, their occurrence in food and aquatic products remains a public health concern because infection may lead to serious outcomes, particularly among immunocompromised individuals or those with wound exposure (El-Zamkan et al., 2023). *Vibrio alginolyticus* is a naturally occurring marine bacterium and an emerging pathogen of both humans and animals; it is also recognized as a major cause of vibriosis in some settings. As an opportunistic pathogen, it is often associated with individuals with weakened immunity or open wounds, and its rapid progression and high mortality in severe infections make it clinically significant (Norfolk et al., 2023; Cai et al., 2024). Likewise, *Vibrio fluvialis*, which is commonly associated with coastal environments, has gained attention as an emerging pathogen because of its involvement in diarrheal outbreaks and occasional extraintestinal infections (Ramamurthy et al., 2014).

The increasing occurrence of *Vibrio* related infections worldwide may be linked, in part, to climate change and the associated rise in sea surface temperatures. Over the past century, sea surface temperature has increased by approximately 1°C, creating more favorable conditions for Vibrio growth, persistence, and pathogenicity. Warmer waters enhance bacterial culturability, growth, and virulence-related traits, while also influencing interactions with aquatic hosts and abiotic surfaces. In particular, ocean warming strengthens associations between *Vibrio* species and zooplankton,

which can serve as reservoirs that support bacterial survival and dissemination in marine ecosystems (Vezzulli et al., 2015). These ecological shifts highlight the broader impact of climate change on the epidemiology of Vibrio infections and underscore the need for interdisciplinary research to understand their transmission dynamics.

Recent evidence also shows that climate change is contributing to the spread of waterborne diseases caused by *Vibrio* species, especially in warm, low-salinity waters. Important pathogenic species such as *V. vulnificus*, *V. parahaemolyticus*, and *V. cholerae* are strongly influenced by environmental temperature, with their prevalence increasing as ocean conditions become warmer and more variable. Human cases and outbreaks have risen in regions previously considered low-risk, suggesting that *Vibrio* may serve as an indicator of climate-driven ecological change in marine environments. Given this pattern, addressing *Vibrio* associated risks requires integrated approaches that combine microbiology, genomics, epidemiology, climate science, and oceanography (Baker-Austin et al., 2017).

Table 1. Occurrence of Vibrio species in composite seafood samples collected from three Public Wet Markets in Bukidnon, Philippines.

Market	Sample (Composite)		
	Mud Clam N (%)	White leg shrimp, n (%)	Mud crab, n(%)
Malaybalay	2/3 (66.6%)	2/3 (66.6%)	1/3 (33.3%)
Valencia	3/3 (100%)	2/3 (66.6%)	1/3 (33.3%)
Maramag	3/3 (100%)	0/3 (%)	1/3 (33.3%)

3.2 Antibiogram of *Vibrio* Isolates

The antimicrobial resistance profile of *Vibrio* species isolates in this study reveals critical insight into both current susceptibility patterns and emerging resistance trends, emphasizing the urgent need for ongoing surveillance and targeted interventions. Fifteen *Vibrio* isolates are resistant to ampicillin but susceptible to chloramphenicol and tetracycline. For trimethoprim/sulfamethoxazole and meropenem, one (6.6%) shows intermediate resistance, while fourteen (93.3%) are susceptible. Regarding azithromycin resistance, five isolates (33.3%) are resistant, and ten (66.6%) are susceptible. For cefotaxime, nine (60%) are resistant, four (26.6%) are intermediate, and two (13.3%) are susceptible. For ciprofloxacin, two (13.3%) are resistant, five (13.3%) are intermediate, and eight (53.5%) are susceptible (Table 2). Additionally, ten (66%) isolates demonstrate a multiple-drug resistant (MDR) pattern.

Table 2. Antibiogram profile of fifteen *Vibrio* isolates

Antibiotic class	Subclass	Antibiotics	*Vibrio* isolates		
			Susceptible (n, %)	Intermediate (n, %)	Resitant (n, %)
Beta-lactam	Penicillin	Ampicillin	0	0	15 (100%)
	Carbapenims	Meropenem	14 (93.3%)	1 (6.6%)	0
	Cephems	Cefotaxime	2 (13.3%)	4 (26.6%)	9 (60%)
Macrolides		Azithromycin	10 (66.6%)	0	5 (33.3%)
Phenicol		Chloramphenicol	15 (100%)	0	0
Folate pathway inhibitors		Trimethoprim-sulfamethoxazole	14 (96.3%)	1 (3.7%)	0
Fluoroquinolones		Ciprofloxacin	11 (40.8%)	2 (7.4%)	2 (7.4%)
Tetracycline		Tetracycline	15 (100%)	0	0

Similar research on *Vibrio* species isolated from green mussels (*Perna viridis L.* 1758) in Bacoor Bay, Cavite, by Tabo (2015) showed resistance to various antibiotics such as ampicillin, nalidixic acid, tetracycline, and co-trimoxazole. Another study by Sun (2024) also reported strains of *Vibrio alginolyticus* isolated from seafood and freshwater sources in China exhibited resistance to multiple antibiotics, including beta-lactams, tetracyclines, and fosfomycin, which was linked to the presence of *blaCARB*, *tet*, and *fos* genes. From 2005 to 2007, Oh (2011) investigated *V. parahaemolyticus* isolates from farmed fish species such as olive flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes schlegeli*), red sea bream (*Pagrus major*), and sea bass (*Lateolabrax japonicus*) and found these isolates to be resistant to ampicillin.

The findings of Hernandez-Robles (2016) further enrich the discussion by demonstrating that *Vibrio alginolyticus* strains isolated from oyster samples in a food market in Mexico City exhibited high levels of antimicrobial resistance. Specifically, over 90% of these strains were resistant to beta-lactam antibiotics, with resistance rates of 60% to cephalotin, 45% to amikacin, 16% to cefotaxime, and 10% to pefloxacin. Importantly, all isolates remained susceptible to ceftriaxone. These *V. alginolyticus* strains exhibited multidrug resistance, paralleling the patterns observed in other geographic regions like Hong Kong, US, and China reinforcing the global challenge posed by resistant *Vibrio* species in seafood sources. This data emphasizes the necessity for continuous antimicrobial resistance monitoring and the promotion of prudent antibiotic use in aquaculture and seafood handling to protect public health.

Including the findings of Kang (2016), *Vibrio alginolyticus* strains isolated from oysters in the West Sea coastal regions of Korea exhibited resistance to ampicillin, vancomycin, and cephalothin. All isolates also showed intermediate resistance to erythromycin and rifampin. Similarly, studies in the Eastern Adriatic Sea highlighted that *V. alginolyticus* isolates were more frequently found in the gills than on the skin of fish and demonstrated statistically significant high susceptibility to flumequine, chloramphenicol, and oxytetracycline (Damir et al., 2013). Furthermore, *Vibrio alginolyticus* strains isolated from mussels in Italy were reported to carry plasmid DNA, with all isolates resistant to ampicillin, carbenicillin, and streptomycin (Ripabelli et al., 2003). These results demonstrate the widespread occurrence of antimicrobial resistance in *V. alginolyticus* across diverse geographical regions and aquatic sources, highlighting the role of plasmids in the dissemination of resistance genes and the variability in susceptibility profiles that depend on the aquatic environment.

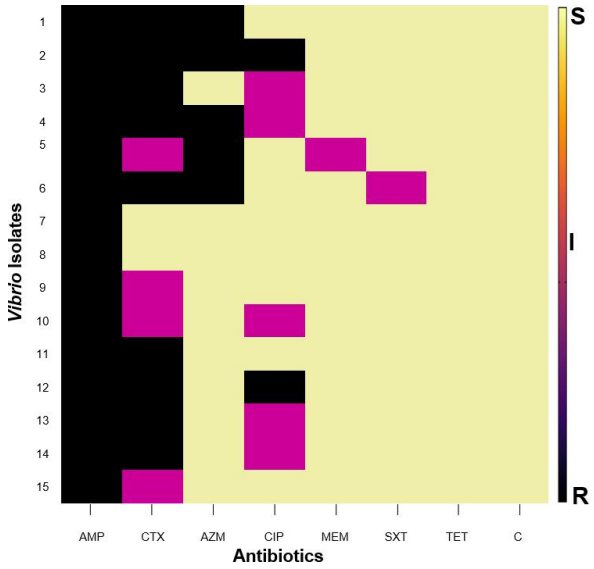

Figure 2. Heat map showing the antimicrobial resistance profile of the individual fifteen *Vibrio* species isolated from seafood samples. Antibiotics are abbreviated as follows: ampicillin (AMP), cefotaxime (CTX), azithromycin (AZM), ciprofloxacin (CIP), meropenem (MEM), trimethoprim/sulfamethoxazole (SXT), tetracycline (TET), and chloramphenicol (C).

3.3 Antimicrobial Resistance Phenotypes and Multiple Antibiotic Resistance Index (MARI) of *Vibrio* Species

Table 3. Multiple Antibiotic Resistance (MAR) Index of *Vibrio* isolates.

Resistant Phenotype	Number of Isolates	Number of antibiotic classes tested	MAR Index	Interpretation
AMP	5	6	0.1	Low-Risk
AMP, CTX	4	6	0.3	High-Risk
AMP, AZM	1	6	0.3	High-Risk
AMP, AZM, CTX	3	6	0.5	High-Risk
AMP, CTX, CIP	1	6	0.5	High-Risk
AMP, AZM, CTX, CIP	1	6	0.6	High-Risk

The *Vibrio* isolates display six resistance phenotypes (Table 3), while the MAR index ranges from 0.1 to 0.6, with isolates classified as "high risk" (MAR index ≥ 0.2) (Table 3). The Multiple Antibiotic Resistance (MAR) index analysis as described previously by Gxalo (2021), isolates from different public markets and shellfish sources reveals notable variations in antibiotic resistance profiles. Among the three municipalities studied, Valencia samples exhibited MAR index values ranging from 0.14 to 0.6, indicating a relatively higher level of antibiotic

exposure or selective pressure in this area. In contrast, Maramag samples showed a 0.1 MAR index. Malaybalay samples with values ranging from 0.1 to 0.5 MAR index.

Considering the MAR indices across various shellfish samples, mud clams showed a range of 0.1 to 0.6, which is slightly higher than the values observed in white leg shrimps and mud crabs, ranging from 0.1 to 0.5. This indicates that mud clams may be exposed to or accumulate higher levels of antibiotic-resistant bacteria, likely due to their habitat or feeding behaviors that increase contact with contaminated sediments or water. These findings highlight the variability of antibiotic resistance in *Vibrio* spp. or other bacterial isolates, depending on geographic location and sample source.

The presence of high MAR index values among *Vibrio* isolates indicates where antibiotics where greatly utilized (Gxalo, 2021). The predominant AMP, CTX resistance phenotype suggests significant resistance to β-lactam antibiotics, particularly ampicillin and cefotaxime. The highest MAR index (0.6) was linked to identified as *Vibrio* sp. is resistant to four antibiotics: AMP, AZM, CTX, and CIP. This multi-drug resistant profile is especially concerning, as it includes resistance to ciprofloxacin, a widely used fluoroquinolone and a critical drug for cholera treatment (Khan et al., 2015). Its resistance indicates emerging treatment challenges. Furthermore, isolates resistant to AMP, AZM, and MEM with MAR indices of 0.37 highlight the co-resistance trend, especially involving azithromycin (AZM), a first-line treatment for cholera. The detection of resistance to MEM (meropenem), a last-resort antibiotic, is concerning and warrants further investigation. Patients infected with meropenem-resistant organisms have higher risk of treatment failure and death compared with infections by susceptible strains (Judd, 2016).

Studies such as those conducted by Morgado (2024) and Adesiyan (2022) have reported similar ranges of Multiple Antibiotic Resistance (MAR) indices and comparable resistance profiles in *Vibrio* isolates. Morgado (2024) identified *Vibrio parahaemolyticus* isolates exhibiting MAR indices as high as 0.38, with predominant resistance to ampicillin and other β-lactam antibiotics, closely matching the frequent AMP, CTX resistance phenotype and the MAR index range of 0.25 to 0.50 observed in this study. Similarly, Adesiyan (2022) documented MAR indices ranging from 0.11 to 0.72 among *Vibrio* isolates sourced from freshwater environments, with a remarkable 97% of isolates categorized within the high-risk group (MAR ≥ 0.2).

Further supporting these observations, Haque (2012) revealed multidrug resistance in *Vibrio cholerae* isolates, with MAR indices reaching up to 1.0, exhibiting resistance to ampicillin, cefotaxime, azithromycin, and ciprofloxacin. This aligns with our detection of resistance phenotypes involving these antibiotics, including the alarming emergence of ciprofloxacin and meropenem resistant strains, signaling potentially significant challenges in treatment. In marine settings, Su (2022) found that approximately 72% of *Vibrio* isolates recovered from oriental hard clams were multi-drug resistant, with MAR values ranging between 0.18 and 0.31. The predominant resistance was noted against ampicillin and related β-lactams. Additionally, their study emphasized a correlation between co-resistance and environmental pressures such as heavy metal contamination, complementing our discussion on the influence of habitat and external factors on the antibiotic resistance profiles and MAR indices.

Moreover, Deng (2020) reported widespread multidrug resistance among Vibrio species, with over 64% of strains resistant to three or more antibiotic classes and MAR indices ranging from 0.00 to 0.60. A higher MAR index approaching 1 indicates a greater degree of antibiotic resistance, whereas a lower MAR index near 0 indicates little or no resistance (Isaiah, 2025). These data similarly highlight the presence of high-risk contamination sources and further bolster our argument regarding the public health implications posed by multidrug-resistant *Vibrio* strains and the critical need for ongoing MAR index monitoring across clinical and environmental isolates. Together, these studies provide strong and supportive evidence backing our observations of MAR index ranges (0.1–0.6), β-lactam resistance (AMP, CTX), the involvement of azithromycin and ciprofloxacin in multidrug resistance, and variability in resistance patterns based on geography and sample source. Multi-drug resistant Vibrio species are an increasing concern across aquatic environments, where they threaten aquaculture productivity and public health. This underscores the need for continued surveillance and targeted antibiotic stewardship programs, including routine resistance monitoring, responsible antimicrobial use in aquaculture, and strengthened biosecurity measures. These findings reaffirm the urgent necessity for establishing routine surveillance frameworks and prudent antibiotic use policies in aquaculture. Monitoring MAR indices offers a valuable epidemiological approach and assess the potential health risks associated with *Vibrio* sp. isolates from both clinical and environmental sources.

CONCLUSION

This study confirms that *Vibrio* isolates are highly present in the shellfish sold in Malaybalay, Valencia, and Maramag public markets in Bukidnon, Philippines, posing potential food safety risks. The findings underscore the importance of enhanced management practices to mitigate *Vibrio* associated health risks in the region.

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