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Prevalence and Antibiotic Resistance Patterns of *Vibrio spp.* in Assorted Retail Shrimp Products Sold in General Santos City and Sarangani Province

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Abstract

Antimicrobial resistance (AMR) in the farm-to-fork chain is a pressing public health threat, particularly in aquaculture, where antibiotics are used to boost production. Owing to the high consumption of shrimp in the Philippines, AMR in this economically important aquaculture product is of special concern. However, the risks of antibiotic-resistant bacterial contamination are poorly understood in key production areas, requiring urgent attention to protect public health. To address this concern, this study investigated the occurrence of antibiotic-resistant *Vibrio* in various shrimp products sold by retail vendors in two major shrimp production areas (Sarangani Province and General Santos City). From 35 shrimp samples purchased from ambulant and permanent retail stores, a total of 60 isolates were generated, of which 24 were identified via the VITEK 2 Compact System Gram-negative identification protocol as *V. parahaemolyticus* (n = 10), *V. fluvialis* (n = 5), *V. alginolyticus* (n = 8), and *V. metschnikovii* (n =1). Moreover, while several *Vibrio* isolates had resistance to one or two antibiotics, no isolate manifested multidrug resistance. The predominant resistance phenotype was Amp^R (ampicillin resistant; 54.2%), followed by Amp^R-Tet^R (ampicillin-tetracycline resistant; 16.7%), Amp^R-$Ceft^R$ (ampicillin-ceftiofur resistant; 4.2%), and Amp^R-Cef^R-$Ceft^R$ (ampicillin-cefazolin-ceftiofur resistant; 4.2%). In addition, we recovered other potentially pathogenic isolates from the shrimp samples, such as *Aeromonas* spp., *Chromobacterium violaceum, Sphingomonas paucimobilis, Shewanella putrefaciens, Pseudomonas stutzeri, Photobacterium damselae*, and *Acinobacter haemolyticus.* Our findings raise concerns about the high incidence of ampicillin resistance among *Vibrio* isolates, which can lead to the development of antibiotic resistance among pathogens infecting cultured shrimps and human consumers. This underscores the need for continuous surveillance of antimicrobial usage in shrimp aquaculture to mitigate the spread of AMR and improve food safety controls, protecting consumers from the transfer of antibiotic-resistant *Vibrio* spp. through the food chain.

Keywords: *antibiotic-resistant bacteria, antimicrobial resistance, food safety, shrimp, Vibrio*

The shrimp industry in the Philippines has substantially contributed to the nation's economic growth by providing employment opportunities, food security and export revenues [1]. The Pacific Whiteleg shrimp (*Penaeus vannamei*) was first introduced in the Philippines in the 1970s [2]. Typically farmed in brackish waters, *P.vannamei* is one of the penaeid shrimp species that has contributed to 92% local consumption [1]. From 2017 to 2020, SOCSARGEN and Central Visayas significantly surpassed Western Visayas in the production of *P.vannamei* [1]. This species is preferred over *P. monodon,* another penaeid species farmed in the country, due to its high market value and short-term culture [3].

The increasing demand for animal protein has intensified shrimp production in the Philippines, leading to multiple environmental problems in the aquaculture sector [4], including the accumulation of antibiotic residues in the sediments in production systems [5, 6]. This raises the public health concern of antimicrobial

resistance (AMR) development, which is exacerbated by the irrational use of antibiotics in shrimp aquaculture [6].

In recent years, the prevalence of antibiotic -resistant bacteria (ARB) in the food production chain has posed food safety risks for consumers [7]. Shrimps in their aquaculture environments are susceptible to *Vibrio* infection or vibriosis [8]. The documented presence of *Vibrio* species, including antibiotic-resistant strains in seafood products from the Philippines, such as those found in Bacoor Bay, underscores the risk of bacterial contamination and highlights growing public health concerns over foodborne outbreaks and AMR transmission along the food chain [9-11].

Foodborne bacterial pathogens are capable of developing into ARB when their aquatic environment has been contaminated with antibiotic residues stemming from the continuous use of antimicrobials [12]. Moreover, the AMR genes of ARB can be transferred to human microflora through horizontal gene transfer via the consumption of contaminated food [13, 14]. Owing to the rising risk of AMR in aquaculture settings, improper food handling, poor hygiene and sanitation, and weak food safety measures can lead to cross-contamination of antibiotic-resistant *Vibrio* in food production [15].

Shrimp is one of the most valuable seafood commodities in Southeast Asia [16]. In the SOCSARGEN Region, General Santos City and Sarangani Province are two key areas, particularly recognized for their vibrant seafood trade, including shrimp production [1]. Despite the intensive shrimp farming activities in these regions, there is limited information on the prevalence of AMR *Vibrio*. This lack of knowledge is a critical gap that may contribute to the increased risk of developing and spreading AMR throughout the farm-to-plate continuum.

To address the issue of AMR in shrimp, this study aimed to investigate the occurrence and antibiotic resistance phenotypes of *Vibrio* isolated from fresh raw, semi-processed, and cooked shrimp sold by ambulant and permanent retail vendors in General Santos City and Sarangani Province. Samples were bought from vendors between June and August 2024, and isolation was performed using conventional microbiological methods. AMR phenotyping was based on the minimum inhibitory concentration generated in an automated antimicrobial susceptibility testing platform.

Materials and Methods

Collection of Shrimp Samples

Shrimp samples were purposively collected from retail vendors in General Santos City and Sarangani Province, Philippines, both key areas for shrimp production and trade. This approach targeted locations in SOCSARGEN Region that are representative areas with high shrimp production, with the distribution of geographical sampling sites across General Santos City and Sarangani Province (Figure 1). A total of 35 shrimp samples were purchased from 35 retail stores. Each store was represented as a vendor, either ambulant retail vendor or ARV (n=13), or permanent retail vendor

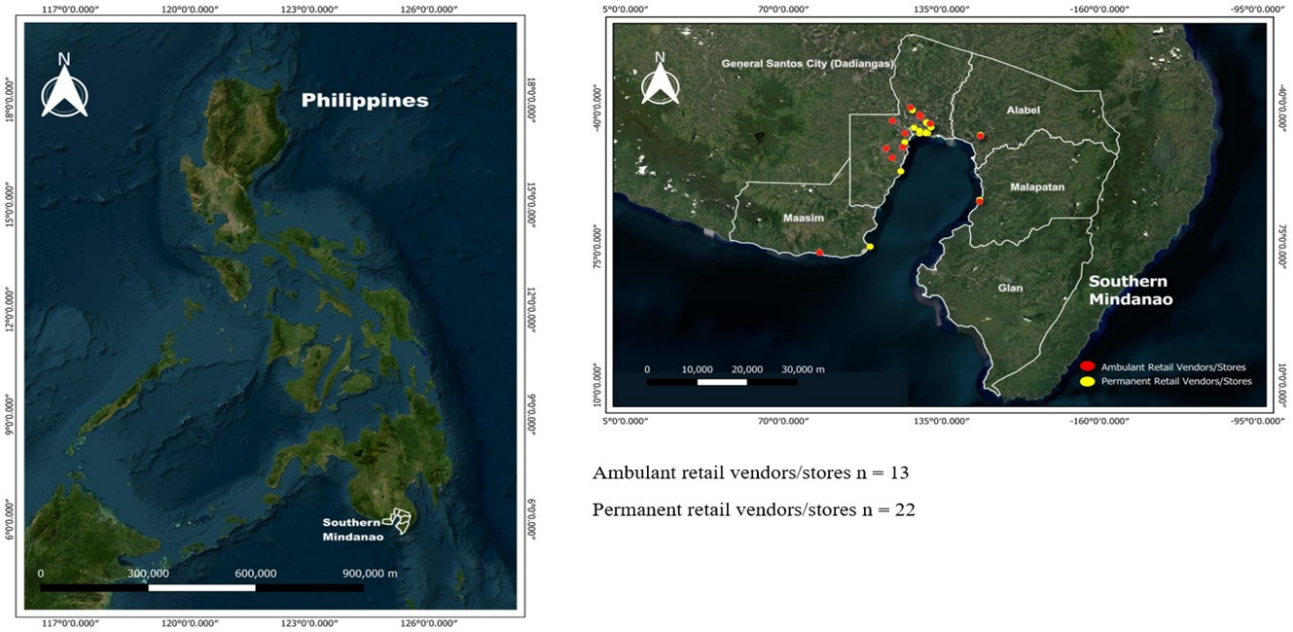

Figure 1. Map of the SOCSARGEN Region, Philippines showing the geographical distribution of sampling sites for retail shrimp products in ambulant (red dots) and permanent retail vendors/stores (yellow dots) in General Santos City

or PRV (*n*=22). A purposive sampling method was used to predetermine sampling locations of retail stores that met certain criteria, as presented in Table 1. Furthermore, the retail shrimp samples were classified into three types of shrimp commodities [17-20], as described in Table 2, namely, (i) fresh–raw shrimp, (ii) semi-processed, and (iii) cooked shrimp.

Isolation and Detection of *Vibrio* Using PNS ISO 21872-1:2022 Protocol

One bag each of fresh raw and cooked or semi-processed shrimp product was bought from each retail store. The net weight of each bag ranged from 375 g to 1000 g, depending on the packaging, and was considered a "sample." Samples were individually packed in sterile autoclavable bags, labeled with product details, and transported in an insulated chiller with ice. Samples were delivered to the Microbiology Analytical and Research Laboratory, College of Natural Science and Mathematics, Mindanao State University – General Santos (MSU – General Santos) in Barangay Fatima, General Santos City for processing and isolation of *Vibrio* within 24 hours of collection.

The Philippine National Standard (PNS) ISO 21872-1:2022 method, an adoption of the ISO 21872-1:2017 method entitled "Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. — Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus, Vibrio cholerae,* and *Vibrio*

Table 1. Selection criteria for identifying permanent and ambulant retail stores included as sampling sites in General Santos City and Sarangani Province.

Criterion	Inclusion	Exclusion
Target Retail Stores	Highly involved in any of the following retail activities • Food preparation, processing, delivery/distribution, & storage. • Implement hygiene practices in the kitchen & display area. • Sells food to the public. • Collaborate with prospect buyers & suppliers. • Provide direct assistance/manage the store activities. • Conduct food safety training to employees. • Other relevant activities involved in retailing activity.	No undertaking of relevant activities mentioned in the inclusion criteria.
Geographic location	SOCSARGEN areas only; General Santos City and Sarangani Province	Conduct of retail activities outside General Santos City and Sarangani Province.
Selling commodity	Produce and sell the following type of shrimp products on the day of the sample collection such as: (i) fresh raw; (ii) semi-processed; and (iii) cooked shrimp	Unable to produce or do not sell the following shrimp products.
Qualification of a permanent retail store and/or ambulant retail store as a mode of selling	*Ambulant retail store* • Conduct of retail operations wherein shrimp products are sold on the street from pushcarts or baskets or balance poles, or from stalls having fewer than four permanent walls; or mobile vendors who walk or bicycle through the streets as they sell. • Conduct of retail operations from semi-fixed stalls, like folding tables that are removed from the streets and stored overnight. *Permanent retail store* • Having a permanent built-up structure and fixtures fixed in a position from which to sell such as retail grocery and seafood shop where products are offered to sale. • Conduct of retail operations in a partially open commercial complex with vending stall organized in rows such as the wet markets.	Does not meet any of the specified criteria/conditions required for inclusion in the study.

Table 2. Classification of samples into types of shrimp commodities sold in the retail markets of General Santos City and Sarangani Province.

Type of shrimp commodity		Description
Raw–fresh shrimp	*Whole shrimp*	All body parts of the shrimp are sold intact. Shrimps that have been freshly caught and have not undergone any preservation treatment, except for chilling [17].
Semi-processed shrimp	*Frozen shrimp*	Refers to whole, headless with the shell on, peeled, peeled and deveined, cooked, or uncooked which have undergone a freezing process that lowers their temperature to -18°C (0°F) to preserve their quality and are maintained at this temperature [17].
	Headless shrimp	Head is detached from the shell & tail; shell & tail remain intact; the vein is removed from the severed head portion [17].
	Peeled & Deveined	The vein and the shell are all removed [17].
	Peeled & Undeveined (PUD)	Shell is removed but the head is intact.
Cooked shrimp	*Boiled shrimp*	Refers to certain body parts may be removed while others remain intact. Shrimp is in orange color as an indicator that product was cooked prior to selling. The shrimp is cooked in boiling water [18].
	Fried shrimp	Refers to certain body parts may be removed while others remain intact. The shrimp is prepared fried in heated cooking oil [18].
	Salted shrimp	Certain body parts may be removed while others remain intact. It is salted cooked in a variety of ways [18].
	Baked shrimp	Certain body parts may be removed while others remain intact. The shrimp is cooked via oven and/or microwave [19].
	Seasoned shrimp	Shrimp is cooked and seasoned with variety of flavors [20].

vulnificus" [21] was followed to isolate suspected *Vibrio* sp. The protocol is outlined in Figure 2. Incubation temperature for primary enrichment depended on the sample type or product state, and was set at 37°C±1°C and 41.5°C±1°C for fresh, raw, and semi-processed shrimp, or 37°C±1°C only for cooked shrimp. Following the protocol's guidelines, three primary selective media from TM Media (Delhi, India) were used: Alkaline Saline Peptone Water (ASPW) for primary enrichment, Thiosulfate Citrate Bile Salt Sucrose Agar (TCBSA), and Saline Nutrient Agar (SNA) for primary and secondary isolation.

A 25-g composite sample of the shrimp product was prepared by finely mincing several pieces of shrimps from a single vendor using a sterile knife, including portions from the middle part (hepatopancreas) and head. The homogenized sample was transferred into 225 ml of ASPW for the initial suspension. After an incubation period of 6 h ± 1 h at 41.5°C ± 1°C for fresh products or 37° C ± 1°C for all product types, a 1-ml aliquot was plated out from the enrichment culture on TCBSA, then incubated at 37°C ± 1°C for 24 h ± 3 h. Presumptive colonies exhibited colony morphologies based on sucrose metabolism, wherein sucrose-fermenting species produce yellow colonies, while non-sucrose-fermenting species produce green colonies [22]. Both yellow and green colonies were further purified by streaking on fresh TCBSA plates. Colony morphology on TCBSA was recorded for presumptive identification. *V. metschnikovii* typically form yellow colonies. *V. parahaemolyticus* produces green colonies. *V. alginolyticus* appears as large yellow colonies, while *V. fluvialis* shows yellow colonies [23-25]. Yellow and green colonies that were circular, raised to a convex elevation, had an entire margin [22], and exhibited shiny and smooth texture [26], along with Gram-stain results showing curved rod-shaped, pink cells [27], were identified as presumptive *Vibrio*.

Presumptive colonies were further sub-cultured onto SNA. Colonies that appeared on the SNA plates after incubation were used to develop stock pure cultures on SNA slants incubated at 37°C for 24 h ± 3 h. SNA slants were

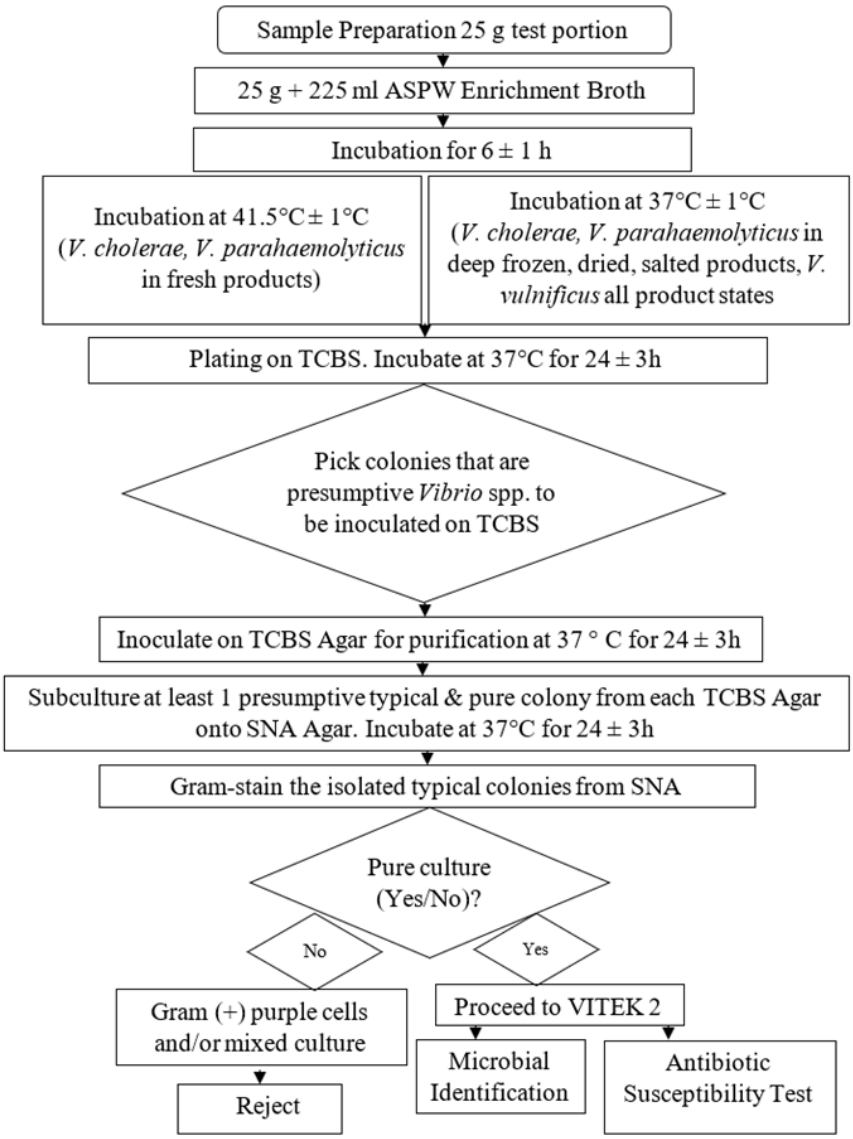

Figure 2. Workflow for the isolation of *Vibrio* from raw and processed shrimp food products using the Philippine National Standards-adopted protocol ISO 21872-1:2022, entitled "Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. — Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*."

then overlaid with sterile mineral oil and stored at 4°C. These presumptive *Vibrio* sp. isolates were subjected to automated biochemical assays for confirmation of identity and to antibiotic susceptibility testing in the VITEK2 Compact System 9.0 (bioMerieux, Marcy l'Etoile, France). All microbiological methods were handled in a biological safety cabinet (BSC) Class II Type A2 (MedFuture Biotech Co., Ltd., Jinan, China). Furthermore, all samples that have come in contact with cultures or microbiologically processed food samples were decontaminated by autoclaving at 121°C for 15 min (15 psi) before disposal.

Bacterial Identification and Antibiogram Using VITEK 2 Compact System

The VITEK2 Compact System accurately identifies bacterial species and profiles antimicrobial resistances for clinical and food testing [28-30]. In this study, the VITEK2 Compact System 9.0 (bioMerieux, Marcy l'Etoile, France) was used for biochemical identification of a total of 60 isolates, including 24 presumptive *Vibrio* isolates, which were the target species in the study. The *Vibrio* isolates were subjected to antibiotic susceptibility testing (AST). The bacterial identification and antibiotic susceptibility test using

VITEK2 Compact System are outlined in Figure 3.

The VITEK2 Gram-negative (GN) identification protocol identifies microorganisms [28] by analyzing enzymatic activities and biochemical reactions in specific substrates, requiring only a minute portion of a colony on a plate [31, 32]. GN cards were acclimatized before use at room temperature. A 3 ml volume of 0.45% saline solution was prepared in a sterile tube. From a bacterial culture grown on SNA plate, 3-5 well-isolated colonies were mixed well in the saline solution to create an ID (identification) suspension. The optical density of this suspension was checked in the VITEK DENSICHECK (bioMérieux. Marcy l'Etoile, France), which had to be at least 0.5 to 0.63 McFarland's value. The GN card was immersed in the ID suspension, verified, and scanned before insertion into the VITEK2 filler

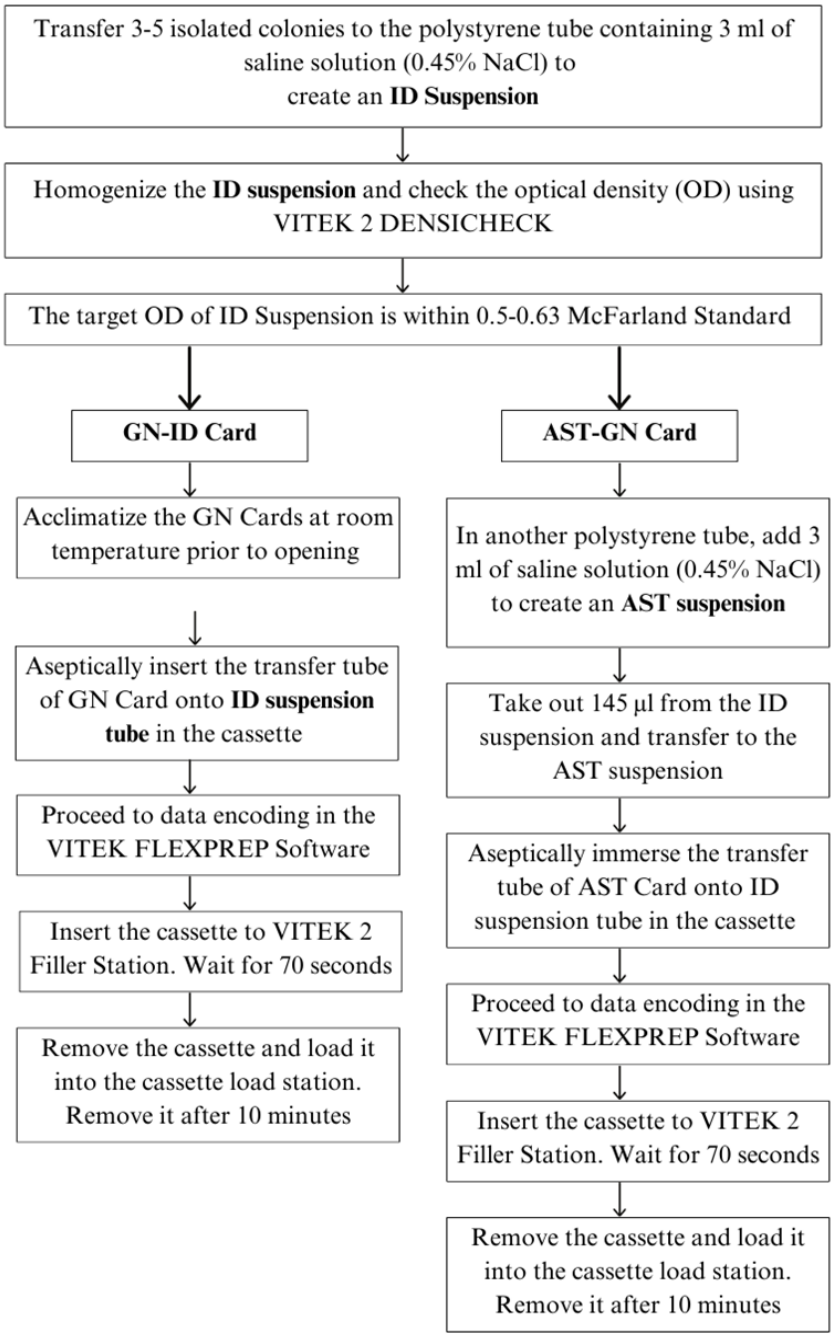

Figure 3. Bacterial identification and antibiotic susceptibility test using VITEK2 Compact System 9.0 (bioMerieux, Marcy l'Etoile, France) of suspected *Vibrio* sp. isolates from shrimp products purchased from vendors in General Santos City and Sarangani.

station. The system incubated the cards, generating results within ten hours or less. The VITEK 2 AST GN-84 and AST GN-96 cards were used to assay the susceptibility of *Vibrio* isolates to various antibiotics (Table 3). A 145 µl-ID suspension was mixed with 3 ml 0.45% saline solution to prepare the AST suspension. One AST suspension each was prepared for AST-GN84 and AST-GN96.

The VITEK2 Automated Compact System identification results are based on its Advanced Expert System (AES) knowledge base [33]. The system computes a percent probability by comparing the set of test reaction patterns against the expected reaction patterns of each organism. Once the reaction pattern closely matches the system-provided pattern, the system generates the percent probability. A higher percent probability indicates a strong match to the expected biochemical reaction pattern of a species or genus in the VITEK 2 AES database.

Table 3. Antibiotics included in the VITEK2 AST-GN84 and AST-GN96 cards for the antimicrobial susceptibility testing of suspected *Vibrio* isolates from shrimp.

Antimicrobial Class	AST-GN96	AST-GN84
β-lactam	Ampicillin	Ampicillin
	Amoxicillin/ Clavulanic Acid	Amoxicillin/ Clavulanic Acid
	Ticarcillin/ Clavulanic Acid	Piperacillin/ Tazobactam
	Cefalexin	
	Cefalotin	
	Cefquinome	
	Ceftiofur	
**Cephalosporin	Cefoperazone	Cefepime
		Ceftriaxone
		Cefazolin
**Monobactams		Aztreonam
**Carbapenems	Imipenem	Meropenem
		Ertapenem
		Imipenem
Aminoglycoside	Gentamicin	Gentamicin
	Neomycin	
Fluoroquinolones	Enrofloxacin	Levofloxacin
	Flumequine	Ciprofloxacin
	Marbofloxacin	
Tetracyclines	Tetracycline	Tetracycline
Nitrofurans		Nitrofurantoin
Lipopeptides	Polymyxin B	
Amphenicol	Florfenicol	
Sulfonamides	Trimethoprim/ Sulfamethoxazole	Trimethoprim/ Sulfamethoxazole

**Sub-classes of β-lactam antibiotics

The information in Table 4 is a summary of the range of percent probabilities of bacterial identification set by the Vitek2 Compact System. The percent probabilities may indicate lower confidence level of identification and may require additional testing for confirmation, such as 16s ribosomal DNA gene sequencing which is ideal for *Vibrio* identification [34].

In this study, the VITEK2 Compact System classified bacterial responses to antibiotics into three interpretative categories: susceptible (S), I (intermediate), and R (resistance) based on breakpoints. The breakpoints are defined as the specific minimal inhibitory concentration (MIC) and are used to categorize isolates as susceptible, susceptible-dose dependent, intermediate, resistant, and non-susceptible to antimicrobial agents by the Clinical and Laboratory Standards Institute [35, 36]. A susceptible (S) phenotype refers to a specific MIC in which the bacterium is effectively inhibited by that antibiotic. An intermediate (IR) phenotype falls between the susceptible and resistant breakpoints. It is "a category defined by a breakpoint that includes isolates with MICs or zone diameters within the intermediate range that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates" [36]. The resistant (R) category has an MIC higher than the established breakpoint range, indicating clinical implications for the treatment regimen. The antibiotic resistance phenotype patterns based on the MIC for each antibiotic in the AST cards were evaluated. Incidence was calculated as the percentage of isolates for a given phenotype.

Results

Distribution of *Vibrio* and Non-*Vibrio* Isolates from Shrimp Samples

The distribution of *Vibrio* and non-*Vibrio* spp. isolates from shrimp product samples purchased from retail vendors is presented in Table 5. A high incidence of *Vibrio* contamination was found in fresh raw shrimps (16 out of 22 samples) and semi-processed shrimp (1 out of 5 samples), but not in cooked shrimp products. Twenty-four (24) *Vibrio* isolates and 36 non-*Vibrio* isolates were obtained in total. Out of the five semi-processed shrimp products examined, only the Nobashi-type yielded isolates, one being *Vibrio* and another one being non-*Vibrio*.

Four *Vibrio* species were identified across the isolates obtained from our shrimp product

Table 4. Identification card range of percent (%) probabilities and their corresponding ID qualifying messages indicating confidence level of the bacterial identity identified in VITEK2 Compact System.

ID Message Confidence Level	% Probability
Excellent	96-99
Very Good	93-95
Good	89-92
Acceptable	85-88
Low Discrimination	2-3 taxa exhibit same biopattern observed from the isolated colonies
Inconclusive or unidentified organism	N/A

samples (Figure 4). *V. parahaemolyticus* emerged as the most prevalent, with ten VITEK 2 GN-confirmed isolates, followed by *V. alginolyticus,* with eight isolates, *V. fluvialis* with five isolates, and *V. metschnikovii*, with a single isolate. Among the *Vibrio* species, *V. alginolyticus* and *V. fluvialis* were identified with VITEK percent probabilities ranging from 88-99%, with confidence levels rated as 'Acceptable to Excellent'. *V. parahaemolyticus* had a probability range of 91-99%, with "Good to Excellent' confidence levels. A single isolate of *V. metschnikovii* showed a 93% probability with a 'Very Good' confidence level (Table 6).

Other non-*Vibrio* species were also isolated from our fresh raw shrimp samples (Figure 4, Table 5). These were *Photobacterium damselae* (3 isolates), *Aeromanas* sp. (16 isolates), *Sphingomonas paucimobilis* (13 isolates), *Chromobacterium violaceium* (1 isolate), *Shewanella putrefaciens* (1 isolate), *Pseudomonas stutzeri* (1 isolate), and *Acinobacter haemolyticus* (1 isolate). *P. damselae* isolates had a 93-96% probability and confidence levels ranging from 'Very good to Excellent' in VITEK 2 GN. *A. sobria* and *A. salmonicida* showed probability ranges of 91-92% with a 'Good' confidence level, and 87-98% with 'Good to Excellent' confidence levels, respectively. For *S. paucimobilis* isolates, the percent probability ranged from 89-97%, with confidence levels of 'Good to Excellent'. Non-*Vibrio* isolates, each represented by a single isolate, including *C. violaceum, P. stutzeri, S. putrefaciens*, and *A. haemolyticus* had percent probabilities ranging from 88-98%, with 'Acceptable to Excellent' confidence levels in VITEK 2 GN (Table 6).

Antibiotic Resistance Profiles of *Vibrio* from Shrimp Samples

The distribution of antibiotic resistant, intermediate resistant, and susceptible phenotypes of the 24 *Vibrio* spp. isolates to individual antibiotics in the VITEK 2 Compact System are shown in Figure 5. Out of the 29 antibiotics tested, the *Vibrio* isolates demonstrated varying resistance phenotypes to the 14 antibiotics, namely, ampicillin, amoxicillin/clavulanic acid, cefazolin, cefepime, ceftiofur, ciprofloxacin, enrofloxacin, gentamicin, imipenem, levofloxacin, marbofloxacin, meropenem, piperacillin/ tazobactam, tetracycline, and trimethoprim/ sulfamethoxazole. All isolates were sensitive to ciprofloxacin (CIP^S), trimethoprim/ sulfamethoxazole (TRI/SUL^S), gentamicin (GEN^S), amoxicillin/clavulanic acid ($AMOXICLAV^S$), imipenem (IMI^S), cefepime ($CEFP^S$), meropenem (MER^S), and piperacillin/tazobactam ($PIPTAZ^S$). For intermediate resistance, cefazolin intermediate

Table 5. Prevalence (distribution and frequencies) of *Vibrio* spp. and non-*Vibrio* spp. in fresh, frozen, and processed shrimp products purchased from the retail markets in General Santos City and Sarangani Province.

Shrimp Product type	No. of Samples	No. of Samples Positive for *Vibrio*	Prevalence[a] *Vibrio* (%)	No. of *Vibrio* isolates	No. of non-*Vibrio* isolates
Fresh	22	16	72.7	23	35
Semi-processed	5	1	20.0	1	1
Cooked	8	0	0.0	0	0
Total	35	17	48.6	24	36

[a]Prevalence (%) = (no. of samples with presumptive *Vibrio* isolate/total number of samples) x100

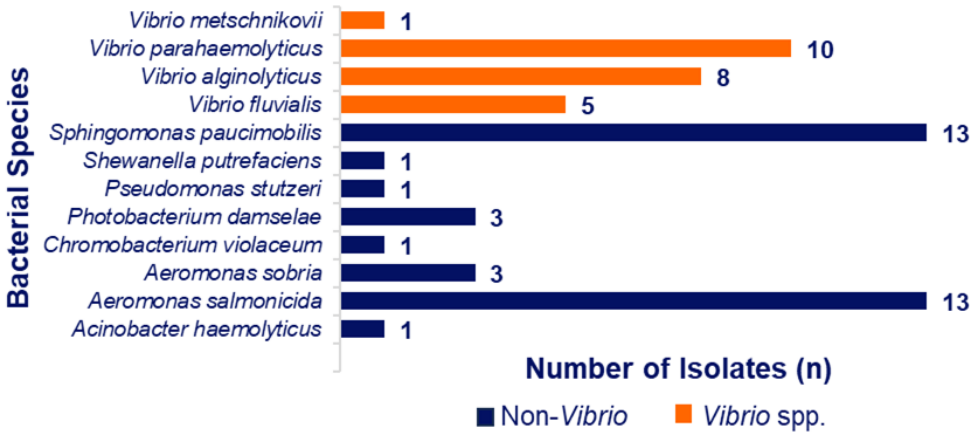

Figure 4. Species distribution of *Vibrio* and non-*Vibrio* isolates from shrimp products purchased from General Santos City and Sarangani Province, with biochemical identification performed in the VITEK2 Compact System 9.0

Table 6. Colony morphology of *Vibrio* isolates observed on Thiosulfate Citrate Bile Salt Sucrose Agar (TCBSA) incubated at 37 °C ± 1 °C for 24 h ± 3 and VITEK GN identification test results of isolates detected in shrimp product samples from General Santos City and Sarangani Province

Isolate Number	Colony morphology on TCBSA; Gram-stain reaction and cell shape	VITEK2 Identification	VITEK Percent Probability	VITEK Confidence Level
1, 2, 3, 4, 5,	Yellow, circular, entire, convex, shiny/smooth colonies; Gram-negative curved rods	*V. fluvialis*	88-99	Acceptable to Excellent
6, 7, 8, 9, 10, 11, 12, 13	Yellow, circular, entire, convex to umbonate, shiny/smooth colonies; Gram-negative curved rods	*V. alginolyticus*	88-99	Acceptable to Excellent
14, 15, 16, 17, 18, 19, 20, 21, 22, 23	Green, circular, entire, convex, shiny/smooth colonies; Gram-negative curved rods	*V. parahaemolyticus*	91-99	Good to Excellent
24	Yellow, circular, entire, convex, shiny/smooth colonies; Gram-negative curved rods	*V. metschnikovii*	93	Very good
25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37	Yellow and green, circular to semi-irregular, entire colonies; Gram-negative rod-shaped	*S. paucimobilis*	89-97	Good to Excellent
38, 39, 40	Green, circular, entire; Gram negative rod-shaped	*A. sobria*	91-92	Good
41, 42, 43, 44, 45, 46, 46, 48, 49, 50, 51, 52, 53	Yellow and green, circular, entire colonies; Gram -negative rod-shaped	*A. salmonicida*	87-98	Good to Excellent
54, 55, 56	Green, circular, entire colonies; Gram-negative rod-shaped	*P. damselae*	93-96	Very good to Excellent
57	Yellow, circular, entire colonies; Gram-negative rod shaped	*C. violaceum*	94	Very good
58	Yellow, circular, entire colonies; Gram-negative rod-shaped	*P. stutzeri*	88	Acceptable
59	Yellow, circular, entire colonies; Gram-negative rod-shaped	*S. putrefaciens*	98	Excellent
60	Yellow, circular, entire colonies; Gram-negative rod-shaped	*A. haemolytcus*	93	Very good

resistance (CEF^I) was the most prevalent, observed in almost 46% of the isolates, followed by ampicillin intermediate resistance (AMP^I) at nearly 21%. Intermediate resistance to levofloxacin, marbofloxacin, and enrofloxacin was observed in 4.2% of the *Vibrio* isolates for each antibiotic.

By analyzing the resistance of *Vibrio* isolates to each individual antibiotic tested, we found that 79% of our *Vibrio* isolates were ampicillin-resistant (AMP^R), rendering this the most common resistance phenotype. This was followed by tetracycline resistance (TET^R, 16.7%), ceftiofur-resistance ($CEFT^R$, 8.3%), and cefazolin resistance (CEF^R, 4.2%). Furthermore, it should be noted that thirteen (13) antibiotics in the AST cards, namely, ticarcillin/clavulanic acid, cefalexin, cefalotin, cefquinome, cefoperazone, neomycin, flumequine, polymyxin B, florfenicol, ceftriaxone, aztreonam, ertapenem, and nitrofurantoin, were unclaimed in the system for antibiogram analysis. This means that the MICs for our *Vibrio* isolates to these antibiotics could not be generated by the VITEK2 Compact system.

Upon investigating phenotype patterns for two or more antibiotics, we found two to three antibiotic resistances in individual isolates. Based on these resistance patterns, a total of six distinct phenotypic antibiotic resistance groups (phenotype groups A to F) were observed among 24 *Vibrio* spp. isolates (Table 7). AMP^R (group A) was the most prevalent resistance phenotype, followed by AMP^R-TET^R (group B), AMP^R-$CEFT^R$ (group C), and AMP^R-CEF^R-$CEFT^R$ (group D). In this study, *V. parahaemolyticus* isolates had three different phenotype groups (A^1, E^2, F), while *V. alginolyticus* produced two (A^2 and E^1). Some isolates of these two *Vibrio* species under phenotype groups E^1, E^2, and F did not show antibiotic resistance to the tested antibiotics but only exhibited intermediate susceptibility (AMP^I, AMP^I-CEF^I). For *V. fluvialis* isolates, only two phenotype groups (B and D) were observed. One isolate of *V. fluvialis* emerged with resistance to three antibiotics (AMP^R-CEF^R-$CEFT^R$, phenotype group D). However, despite showing resistance to multiple antibiotics, this isolate was not considered multidrug-resistant (MDR), as defined by Magiorakos et al. [37], since all three antibiotics are members of the β-lactam class. A single isolate of *V. metschnikovii* exhibited resistance to the β-

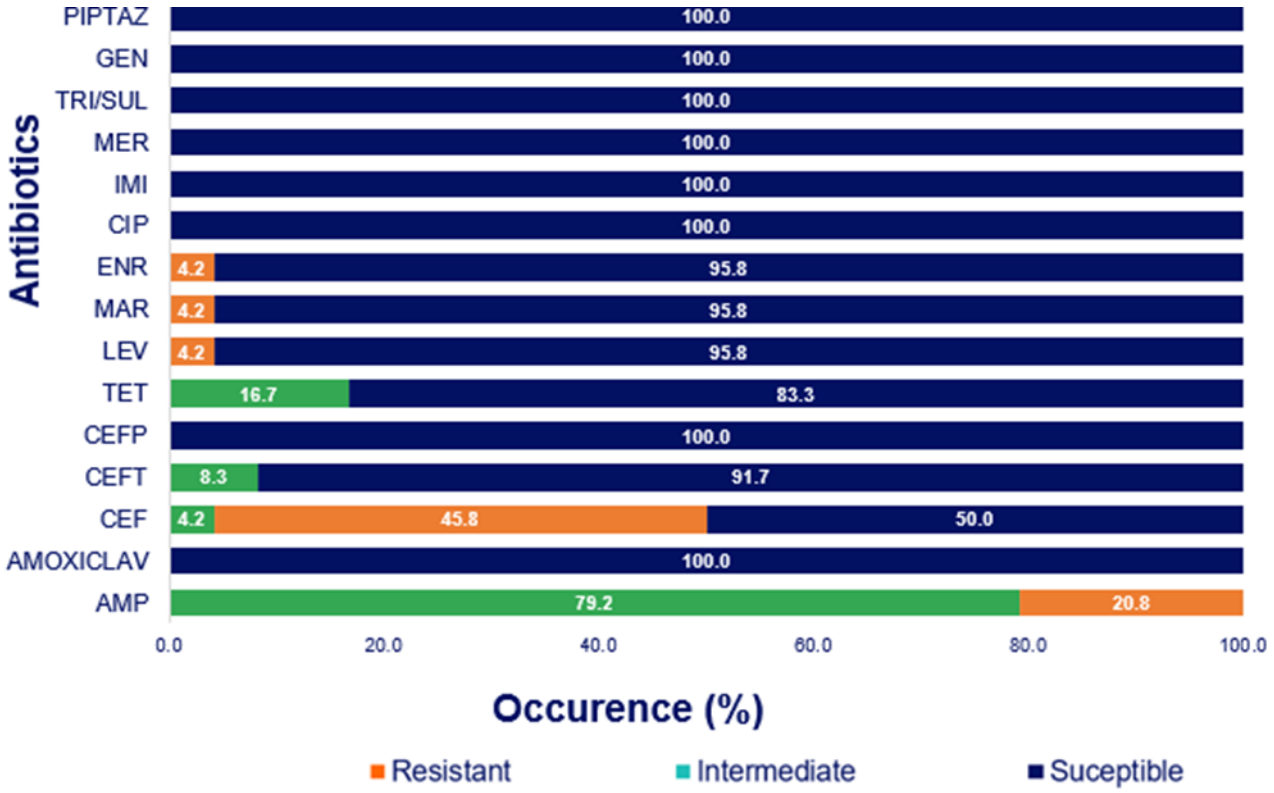

Figure 5. Distribution of the antibiotic resistance phenotypes (resistant, intermediate resistant, and susceptible) generated by the VITEK2 Compact System for 24 *Vibrio* spp. isolates from retail shrimps purchased from General Santos City and Sarangani Province. PIPTAZ - piperacillin/tazobactam, GEN – Gentamicin, TRI/SUL - trimethoprim/sulfamethoxazole, MER – meropenem, IMI – imipenem, CIP – ciprofloxacin, ENR – enrofloxacin, MAR – marbofloxacin, LEV – levofloxacin, TET – tetracycline, CEFP – cefepime, CEFT – ceftiofur, CEF – cefazolin, AMOXICLAV -amoxicillin/clavulanic acid, AMP – ampicillin.

Table 7. Distribution of antibiotic resistance phenotype combinations among *Vibrio* spp. isolates (n = 24) from shrimp products sold in the retail markets of General Santos City and Sarangani Province.

Isolate Number	Identity[a]	Phenotypic Antibiotic Resistance Patterns[b]			No. Isolates (%)	Phenotype Group
		Susceptible	Intermediate	Resistant		
15, 16, 18, 19, 21, 22, 23	*V. parahaemolyticus*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	None	AMP^R	29.2%	A^1
6, 7, 8, 9, 12, 13	*V. alginolyticus*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	None	AMP^R	25%	A^2
1, 2, 3, 5	*V. fluvialis*	CIP^S-$TRISUL^S$-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	None	AMP^R-TET^R	16.7%	B
24	*V. metschnikovii*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-MAR^S	None	AMP^R-$CEFT^R$	4.2%	C
4	*V. fluvialis*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-$CEFP^S$-MER^S-$PIPTAZ^S$	LEV^I-ENR^I-MAR^I	AMP^R-CEF^R-$CEFT^R$	4.2%	D
10, 11	*V. alginolyticus*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	AMP^I	-	8.3%	E^1
20	*V. parahaemolyticus*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-CEF^S-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	AMP^I	-	4.2%	E^2
14, 17	*V. parahaemolyticus*	CIP^S-$TRISUL^S$-TET^S-GEN^S-IMI^S-$AMOXICLAV^S$-$CEFP^S$-LEV^S-MER^S-$PIPTAZ^S$-ENR^S-$CEFT^S$-MAR^S	AMP^I-CEF^I	-	8.3%	F

[a]Identity confirmed using VITEK2 Gram-negative identification cards

[b]R=Resistant, I – Intermediate Resistant, S – Susceptible; dash (-) – not observed; PIPTAZ - piperacillin/tazobactam, GEN – Gentamicin, TRI/SUL - trimethoprim/sulfamethoxazole**,** MER – meropenem, IMI – imipenem, CIP – ciprofloxacin, ENR – enrofloxacin, MAR – marbofloxacin, LEV – levofloxacin, TET – tetracycline, CEFP – cefepime, CEFT – ceftiofur, CEF – cefazolin, AMOXICLAV -amoxicillin/clavulanic acid, AMP – ampicillin.

lactam antibiotics ampicillin and ceftiofur (group C). This pattern of β-lactam ampicillin resistance in *V. metschnikovii* is consistent with findings from multiple studies done in Norway and South Africa [38,39]. This species was also susceptible to amoxiclav and cefazolin, aligning with the findings of Konechnyi et al. [40].

Discussion

Prevalence of *Vibrio* Isolates in Shrimp Samples

Vibrio was detected in majority of the fresh raw shrimp samples and in one semi-processed shrimp sample (Table 5). The occurrence of *Vibrio* in fresh raw shrimp samples may be attributed to minimal processing unlike the other product types, which are typically subjected to a series of processing steps, such as cooking. Fresh raw shrimp retain their microbial flora from their natural tropical habitats [41]. Rising water temperatures in these aquatic habitats, driven by climate change, create favorable growth conditions for *Vibrio* populations to proliferate [42], increasing their prevalence in shrimp and surrounding aquatic systems. Meanwhile, no *Vibrio* was detected in the cooked samples, indicating that the examined products had likely been well-prepared and well-cooked. Low viable microbial counts are expected in cooked samples due to proper temperature control [43]. For the five semi-processed shrimps in this study, only one Nobashi shrimp sample was positive for *Vibrio*. Nobashi shrimp, typically prepared for tempura, undergoes multiple processing steps, including peeling, deveining, stretching, and leaving the tail on [44, 45]. Thus, the detection of *Vibrio* in this product is likely linked to contamination during food preparation and handling due to the extensive processing involved.

Implications of *Vibrio* and Non-*Vibrio* Contamination in Shrimp Samples

Several *Vibrio* species were detected in our shrimp samples (Figure 4). Among them was *V. parahaemolyticus* which turned out as the most common *Vibrio* species in our samples. The tropical warm climate of the Philippines likely promotes the proliferation of microbial density and survival of *V. parahaemolyticus.* Sterk et al. [46] reported that temperature increases the microbial density of Gram-negative bacteria, specifically for *V. parahaemolyticus*. This species is considered highly pathogenic and a leading cause of bacterial infectious diarrhea in several parts of Asia [42]. It is primarily transmitted through the oral route, often via the consumption of raw or undercooked seafood, and through wound exposure to contaminated water. Infections typically occur sporadically along coastal regions, especially during warmer months when bacterial levels in the water increase. While the consumption of contaminated seafood is the most common route of transmission, exposure of open wounds to seawater also poses a risk. Once inside the human body, it can lead to gastroenteritis, wound infections, and in severe cases, septicemia [42]. Another *Vibrio* species isolated was *V. metschnikovii*, which is known to colonize shellfish and thrive in aquatic environments such as freshwater, brackish and marine waters, and sewage [47, 48]. This rare species can also be found in shrimps and other seafoods [49]. In fact, the prevalence of antibiotic-resistant *V. metschinikovii* has been reported in retail shrimp collected in Northern California [50]. Furthermore, it is a water-transmitted pathogen known to cause infections in humans [40] via ingestion of contaminated fish or shellfish, exposure to contaminated water, bacteremia, pneumonia, post-operative wound infection, and infected leg ulcers [49]. Meanwhile, *V. alginolyticus* and *V. fluvialis* thrive abundantly in warm waters above 18°C during the summer season, which implies that these species are temperature-dependent [51-53]. Patients infected with *V. fluvialis* may present with fever, diarrhea, abdominal cramps, and nausea [52], while *V. alginolyticus* is an opportunistic pathogen that causes ear and soft tissues infections in immunocompromised patients [53]. The occurrence of these *Vibrio* species in our samples emphasizes the risk of *Vibrio* contamination in retail shrimp products, particularly in tropical regions like the Philippines, where foodborne outbreaks linked to raw shrimp consumption have been reported [54, 55]. Consumption of undercooked shrimp products contaminated with these *Vibrio* species can result in foodborne illness [56], and may be a result of poor food handling, such as improper separation of raw shrimp and cooked food [57].

The detection of *Vibrio* in retail shrimp products highlights the importance of ensuring microbiological safety of shrimp for domestic consumption [15]. Once *Vibrio* enters the food production chain, the transmission of this foodborne pathogen may lead to the formation of protective biofilms on various food contact surfaces in the food processing areas and cross-contamination, posing food safety risks [15, 58]. Furthermore, the formation of resistant biofilms by the foodborne pathogen *Vibrio* may reduce the effectiveness of cleaning and sanitation procedures, consequently amplifying the AMR contamination route and increasing the food safety risk of disinfection failure. In the absence of proper sanitation protocols, contaminated seafood products may enter the food supply chain, posing

significant health hazards to consumers [58].

Occasional non-*Vibrio* opportunistic pathogens were also detected in the shrimp samples, indicating that the selective medium used (TCBSA) can support the growth of other Gram-negative bacteria [59]. The isolation of various species underscores the importance of comprehensive monitoring not only for *Vibrio* but also for non-*Vibrio* pathogens in shrimp food products for enhanced food safety and reduced public health risks [60]. *Vibrio* sp. and non-*Vibrio* sp. may be present altogether in shrimp food samples due to cross-contamination from shared environments [61], poor handling [62], and inadequate sanitation [15].

All non-*Vibrio* isolates obtained from our shrimp samples are known to be opportunistic pathogens in humans. *Photobacterium damselae,* formerly *V. damsela,* was reclassified to *P. damselae*, due to distinct genetic and phylogenetic characteristics [63]. This is known to cause necrotizing fasciitis and other wound infections due to handling of contaminated seafood [64,65]. A previous study reported the predominance of *P. damselae* in Chinese retail markets, with a prevalence rate of 20.3% [66]. Another opportunistic human pathogen isolated from the samples was *Aeromonas* sp., which is linked to diarrhea, infections in immunocompromised individuals, wound infections, and septicemia [67]. It has been reported previously that *Aeromonas* contaminated 72% of fish and shrimp [68]. The prevalence of *Aeromonas* sp. in marine-associated seafood includes detection in pre-frozen shrimps and ready-to-eat shrimp cocktail [67].

Thirteen (13) isolates identified as *Sphingomonas paucimobilis* were also detected in fresh raw shrimp samples. This Gram-negative, aerobic, motile, and non-fermenting bacillus [69], found in water reservoirs and soil, can cause infections in immunocompromised individuals, including soft tissue infections [70], bacteremia, septicemia, and septic arthritis [71]. Furthermore, *S. paucimobilis* was detected in seafood products obtained from retail fish market in the United Arab Emirates [72]. Its presence highlights the need for proper food handling practices, regular microbiological assessments, and identification of contamination pathways to safeguard food safety [72].

Another bacterial isolate identified as *Chromobacterium violaceum* is considered a rare, highly fatal, Gram-negative bacterial pathogen [73] found in our shrimp samples. This environmental microorganism is widely distributed in tropical regions, particularly in soil and aquatic environments [74]. It is associated with life-threatening sepsis, skin infections, and urinary tract infections [73, 75]. The first documented isolation of *C. violaceum* from oyster products was reported by Berebichez-Fridman et al. [76]. Both *Vibrio* sp. and *C. violaceum* share similar environmental conditions, typically thriving in estuarine and marine environments where salinity is a key factor for their survival [77]. The occurrence of *C. violaceum* in oysters, which are often consumed fresh raw poses a significant food safety concern due to its potential to cause severe infections with a low recovery rate [76]. Likewise, a single isolate of *Shewanella putrefaciens* was obtained from a sampled fresh raw shrimp. Previous studies reported the presence of this spoilage microorganism isolated in refrigerated shrimps in China [78,79]. Furthermore, it is an opportunistic pathogen found in marine environments and is linked to human infections such as pneumonia and endocarditis, potentially leading to death [80].

The single isolate of *Pseudomonas stutzeri*, obtained from a fresh raw shrimp sample, has been reclassified as *Stutzerimonas stutzeri* [81]. It is considered an opportunistic pathogen and thrives in a wide range of natural environments, including sewage, soil, and manure [82]. Patients with *P. stutzeri* infections had one or more risk factors, such as underlying illness, history of surgery, superficial infection, and immunocompromised individuals [83]. This species has been reported in a wide range of environments along the farm-to-plate continuum. For instance, it has been identified in *P. vannamei* pond sediments in Vietnam [84], in the surrounding brackish waters where tropical freshwater shrimp are cultivated [85], in seafood products sold at retail markets in Egypt [86, 87], and in *Penaeus monodon* specimens from fish markets in India [88]. Finally, *Acinobacter haemolyticus* isolated from fresh our raw shrimp sample is known to be widely present in soil, human skin, wastewater and food [89]. It is linked to nosocomial infections and exhibits high resistance to drugs [89], requiring early clinical intervention.

Antibiotic Resistance in *Vibrio* from Retail shrimp: Implications for Public Health and Shrimp Aquaculture Practices

Most *Vibrio* isolates (over 70%) were resistant to ampicillin. The high incidence of ampicillin resistance was also reported in seafood in previous studies [43, 90-94], suggesting that this type of resistance is already widespread. In particular, *V. parahaemolyticus* isolates were resistant to ampicillin and showed intermediate resistance to cefazolin. This finding suggests that ampicillin may no longer be an effective antibiotic treatment for associated infections caused by this species. Similarly, prescribing cefazolin should be

carefully considered as we have already seen intermediate resistance in *V. parahaemolyticus* isolates. Widespread resistance to these antibiotics is regarded as challenging for healthcare providers, and may increase the likelihood of prolonged or more severe infections. The incidence of ampicillin resistance in *Vibrio* has been reported to range from 40% to 90% since 1987 [93]. This trend may be linked to the overuse of first-generation antibiotics in the environment, leading to reduced susceptibility and effectiveness in treating *Vibrio* infections [93]. The high incidence of ampicillin resistance observed in this study was likewise observed in *Vibrio* isolates from shrimps collected from shrimp farms in Ecuador [90], retail stores in Northern California [50], and aquacultural sources in Malaysia [93]. This antibiotic resistance pattern suggests that ampicillin resistance may be widespread among *Vibrio* populations across different shrimp production sources. Ampicillin-resistant *Vibrio* in seafood poses significant health risks to consumers, particularly vulnerable populations like those with weakened immune systems. If contaminated seafood is consumed without proper cooking, it may lead to infections that are difficult to treat with common antibiotics like ampicillin.

V. parahaemolyticus isolates remained susceptible to antibiotics tested in this study, including fluoroquinolones, aminoglycosides, and subclasses of β-lactam antibiotics (such as carbapenems and cephalosporins), trimethoprim/ sulfamethoxazole, ceftiofur, and antibiotic combinations like amoxicillin/clavulanic acid and piperacillin/tazobactam. These findings are comparable to the results in the previous studies, which reported that *V. parahaemolyticus* isolates from retail shrimps in Malaysia were susceptible to imipenem, meropenem (carbapenem sub-class), gentamicin, trimethoprim/ sulfamethoxazole [94], while those from retail aquatic products in North China were susceptible to trimethoprim/sulfamethoxazole [92], gentamicin (aminoglycoside), and ciprofloxacin (fluoroquinolones) [91]. In addition, the susceptibility observed in our isolates to these antibiotics is consistent with findings reported in other studies. For instance, susceptibility of *Vibrio* to meropenem and imipenem was also observed in *Vibrio* isolates from rustic environmental samples collected in a province of South Africa [95], while susceptibility to amoxicillin/clavulanic acid, cefepime, and piperacillin/tazobactam were observed for *Vibrio* isolated from retail shrimps in Ecuador [90]. The observed susceptibility may be attributed to limited exposure of the isolates to these antibiotics, potentially due to differences in antimicrobial usage in shrimp aquaculture. Based on these results, these antibiotics may still be effective treatment options for *V. parahaemolyticus* -associated infections.

Ampicillin may not be effective in treating infections caused by *V. fluvialis* and *V. metschnikovii*, as these isolates have exhibited resistance to ampicillin. Konechnyi et al. [40] presented clinical cases of *V. metschnikovii*-associated infections. The study revealed that ampicillin remained one of the antibiotic treatment options for this pathogen in 1992. However, between 2005 and 2014, resistance to ampicillin became more prevalent in clinical cases. Findings from this study, along with previous reported clinical cases [96-100] collectively suggest that antibiotic resistance of *V. metschnikovii* has increased over time, highlighting the need for continued surveillance of antimicrobial usage to guide effective treatment options.

In this study, we also noted the ampicillin-tetracycline (AMP^R-TET^R, phenotype group B) resistance pattern to *V. fluvialis.* This combination of antibiotic resistance phenotype has been observed in previous studies on shrimp and other seafood [91, 101]. The observed resistance pattern may be attributed to the frequent misuse of these first-generation antibiotics in aquaculture [11]. Tetracyclines are widely used treatments in both human and veterinary medicine [12, 102]. However, ARGs (antibiotic resistant genes) conferring resistance to these antibiotics have now been found in aquaculture settings, particularly in sediments [12]. These ARGs could potentially be transferred to other significant pathogens, leading to the spread of AMR which can pose challenges on treatment in clinical settings [50]. Multiple resistance genes in *Vibrio* spp. isolated from retail shrimp were found on mobile genetic elements such as plasmids, facilitating HGT across bacterial species [50]. Furthermore, the ampicillin resistance of *V. fluvialis* isolates in this study aligns with previous findings from *V. fluvialis*-related acute diarrheal cases, where over 90% of isolates showed ampicillin resistance [103]. The ampicillin was commonly used as an empirical treatment for infections caused by this pathogen. However, given the prevalent ampicillin resistance and the lack of well-established treatment guidelines for *V. fluvialis*-associated infections, there is a need for reconsideration on the use of ampicillin in treating *V. fluvialis*-associated intestinal infections [104].

The observed resistance (AMP^R) in the majority of *V. alginolyticus* isolates in this study is consistent with the findings of Tabo et al. [11] based on seafood samples taken from Bacoor Bay, Philippines, where most isolates also exhibited resistance to ampicillin. Again, this indicates a potential challenge in treating infections associated

with these foodborne pathogens [105]. AMR in bacterial pathogens reduces the effectiveness of antibiotics, forcing reliance on stronger or broader-spectrum antibiotics that may be less accessible or have undesirable side effects [106].

The isolation of ARB in retail shrimp in this study and other seafood suggests that the consumption of raw seafood may pose a food safety hazard to consumers. While *Vibrio* infection is treatable with intravenous and rehydration therapy, antibiotic treatment is crucial during outbreaks to mitigate the severity of the health symptoms and reduce the duration of bacterial shedding [11]. The majority of our isolates exhibited resistance to ampicillin, indicating that this antibiotic may be ineffective for treatment. Furthermore, ampicillin is not recommended in shrimp culture treatment regimens and emphasizes the need for a well-defined diagnosis before its use [107]. The persistence of ampicillin resistance is closely linked to anthropogenic factors, due to its frequent use in aquaculture [15].

In this study, while the proportion of isolates exhibiting monoresistance phenotype was higher than those resistant to combinations of two or three antibiotics, it is still important to continuously monitor AMR to predict the potential spread of multidrug resistance, which could evolve into broader resistance patterns over time [108]. The rising incidence of AMR in the clinical ecosystem [109, 110] has also entered the era of AMR dissemination in the farm-to-plate continuum. AMR is a silent pandemic that is projected to cause 1.91 million attributable deaths and 8.22 million associated deaths by the year 2050, with an 80% increase in AMR-related mortality among adults [111]. The limited treatment options and reduced effectiveness of these antibiotics against certain *Vibrio* species may not only complicate treatment strategies but may also increase mortality and morbidity rates in human populations. AMR *Vibrio* in seafood can act as reservoirs of ARGs which have the potential to spread to other bacteria in the environment or the human gut microbiome [13, 15], exacerbating the broader AMR problem. The high prevalence of resistant *Vibrio* isolates observed in this study emphasizes the need for stringent food safety measures along the farm-to-plate continuum. Improved hygiene, proper handling, and cooking practices are critical to reducing contamination risks [15].

The persistence of AMR *Vibrio* remains a pressing issue despite the implementation of regulatory measures such as Fisheries General Memorandum Order No. 2021-001, Series of 2021, issued by the Department of Agriculture - Bureau of Fisheries and Aquatic Resources. Although this directive mandates laboratory analysis of shrimp samples for antibiotic residues, the testing is limited to chloramphenicol and nitrofurans in fresh, chilled, and frozen shrimp [112]. This limited scope may fail to detect resistance to other antibiotics commonly used in aquaculture, potentially allowing antimicrobial resistance to go unchecked. The inadequate monitoring of antibiotic use in shrimp production elevates the risk of AMR, posing a significant threat to consumer health. We hope our findings will prompt policymakers to regulate and monitor the use of antibiotics in aquaculture. Regulatory measures that limit antibiotic misuse are essential to mitigate the development and spread of resistance and to promote alternative disease treatment regimens, such as the use of probiotics and implementation of biosecurity control in shrimp production facilities.

Conclusion

We report the presence of antibiotic-resistant *Vibrio* species in shrimp products from retail markets, with ampicillin resistance being the most prevalent among the isolates. Although individual isolates exhibited resistance to one to three antibiotics, no multidrug resistance was detected. The high incidence of ampicillin resistance among *Vibrio* isolates, along with the detection of multiple potentially pathogenic strains in fresh shrimp samples, suggests that this resistance may be persistent in aquaculture environments. Also, although the non-*Vibrio* isolates were not tested for antibiotic susceptibility, the widespread acquisition of antibiotic resistance among bacteria suggests they may also harbor resistance traits. Consequently, the consumption of undercooked shrimp contaminated with antimicrobial-resistant pathogenic bacteria poses a risk to consumers' health and may lead to infections with limited antibiotic therapy options. Although the number of samples and the geographical scope of this study are not comprehensive, our findings provide baseline insights into the prevalence of AMR in the region's rapidly growing shrimp industry.

The emergence of antibiotic-resistant bacteria (ARB) in shrimp may stem from multiple contributing factors, including the inappropriate use of antibiotics in farming practices and limitations in the enforcement of Good Aquaculture Practices (GAqP). Moreover, contamination during post-harvest processing and retail, such as through inadequate sanitation and cross-contamination, may further exacerbate the spread of AMR *Vibrio*. Although routine monitoring for antibiotic residues is conducted by local regulatory bodies, the results of this study

highlight the potential for continued circulation of AMR *Vibrio* within the food supply chain.

Our detection of antibiotic-resistant *Vibrio* spp. in retail shrimp products underscores the pressing need for robust food control measures to protect consumer health and public safety. These findings raise significant concerns regarding the potential transmission of antimicrobial-resistant bacteria through shrimp, a widely consumed seafood product. Establishing a baseline dataset on the resistance patterns of *Vibrio* in shrimp is crucial for effective AMR surveillance and monitoring. Such data offer critical insights into the dynamics of antibiotic resistance within the farm-to-plate continuum, enabling targeted interventions to mitigate food safety risks, prevent outbreaks, and ensure the integrity of shrimp as a safe, sustainable food commodity for local and international markets.

Addressing this issue requires enhanced and integrated surveillance of AMR throughout the entire production and distribution network—from hatcheries and grow-out facilities to processing centers, and retail markets. Strengthening monitoring systems will be crucial in pinpointing high-risk areas and implementing targeted control measures to address AMR risks and protect public health.

Author's Contribution

This work is a portion of EPD's masteral thesis under the advisership of LER. LER and EPD conceptualized the study, analyzed the data, and wrote the manuscript. EPD procured the samples and performed the laboratory work.

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