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Original Article

Phenotypic and genotypic antibiotic resistance of dominant bacterial isolates from cultured Whiteleg shrimp, *Penaeus vannamei*

Mary Ann Gito[1,3], Kyla Kris De la Cruz[1,4], Ivanne Pearl Guillermo[1,5], Diana Paguntalan[2], Carmelo del Castillo[6], Stephanie Pimentel[7], Christopher Marlowe Caipang[*1,2, 8]

[1]Graduate School, University of the Philippines Visayas, Iloilo City, Iloilo 5000, Philippines.
[2]Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines.
[3]Natural Sciences Department, College of Arts and Sciences, Iloilo Science and Technology University, Iloilo City 5000, Philippines.
[4]Life Sciences Department, College of Arts and Sciences, Central Philippine University, Lopez Jaena Street, Jaro, Iloilo City, 5000, Philippines.
[5]Department of Biology, College of Liberal Arts, Sciences, and Education, University of San Agustin, Iloilo City, Iloilo 5000, Philippines.
[6]Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines.
[7]Fisheries and Food Research Development Center, Capiz State University, Roxas City 5800, Philippines.
[8]National Institute of Molecular Biology and Biotechnology, University of the Philippines Visayas, Miagao, Iloilo 5023, Philippines.

Abstract: Antibiotic resistance in aquaculture poses significant threats to aquatic animal health and may contribute to the spread of antimicrobial resistance in the environment and human populations. This study characterized the phenotypic and genotypic antibiotic resistance profiles of dominant bacterial isolates recovered from cultured whiteleg shrimp (*Penaeus vannamei*) obtained from shrimp farms in Iloilo, Philippines. A total of 150 shrimp samples were processed for bacterial isolation, and dominant isolates were screened for multidrug resistance (MDR) using the Kirby–Bauer disk diffusion method. Based on the antibiotic resistance index (ARI), nine MDR isolates were selected for further analysis. Phenotypic susceptibility testing showed that all MDR isolates were resistant to ampicillin and rifampin. Resistance to ciprofloxacin (55.5%), erythromycin (44.4%), tetracycline (22.2%), chloramphenicol (11.1%), and streptomycin (11.1%) was also observed. Polymerase chain reaction (PCR) detected antibiotic resistance genes associated with chloramphenicol, tetracycline, and quinolone resistance, namely *catB* (44.4%), *tetE* (22.2%), and *gyrA* (11.1%), respectively. Discrepancies between phenotypic resistance patterns and detected resistance genes were observed, indicating phenotypic–genotypic discordance. These findings suggest that additional mechanisms beyond the targeted genes may influence the expression of resistance. The study highlights the complexity of antimicrobial resistance in aquaculture and underscores the value of integrating phenotypic and molecular approaches for effective surveillance and management.

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Introduction

The discovery of antibiotics in the 20th century has been regarded as one of the greatest breakthroughs in medicine. For several decades, antibiotics have been extensively used as an effective intervention in treating bacterial diseases in human medicine, crop and livestock production, and aquaculture operations (Hutchings et al., 2019; Halawa et al., 2024). Antibiotics in aquaculture are primarily used to alleviate the effects of bacterial infections and to prevent disease (Hernández Serrano, 2005). Antibiotic resistance, an emerging global threat, is defined as the ability of bacteria to overcome or counteract the effects of antibiotics designed to inhibit or eliminate their growth (Beceiro and Bou, 2013; Ventola, 2015; Nadeem et al., 2020). Aside from its perilous effects on human health, major economic losses are also projected. By 2050, additional healthcare costs could climb to US$ 1 trillion, and there could be an annual global gross domestic product (GDP) loss of US$ 1 trillion to US$ 3.4 trillion in 2030 (WHO, 2023). The aquaculture sector has emerged as a contributor to the rise and spread of antibiotic resistance. Excessive and imprudent use of antibiotics in aquaculture has driven the rapid emergence and spread of antibiotic-resistant bacterial strains and genes (Chowdhury et al., 2022;

[*]Correspondence: Christopher Marlowe Caipang
E-mail: cacaipang@up.edu.ph
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Mohammed et al., 2025).

Whiteleg shrimp (*Penaeus vannamei*), one of the leading cultured shrimp species in the Philippines, accounts for about 30% of the country's total national shrimp production (Bureau of Fisheries and Aquatic Resources, 2022). Shrimp farms in the Philippines, such as hatcheries and grow-out ponds, use tetracycline, rifampicin, chloramphenicol, nitrofurans, erythromycin, oxolinic acid, and furazolidone to treat bacterial infections. However, the continued use of antibiotics in aquaculture can intensify selective pressures on microorganisms, leading to the emergence of bacterial strains that can withstand antibiotics (Gao et al., 2012).

Detecting antibiotic resistance mainly involves phenotypic and genotypic strategies. The phenotypic resistance method involves detecting resistance through culture-based methods and determining the minimum inhibitory concentration (MIC) of various antibiotics. Commonly used laboratory methods include the Kirby–Bauer disk diffusion test, agar dilution, E-test, and microdilution (Sahoo et al., 2024). On the contrary, genotypic resistance assessment strategies utilize antibiotic resistance genes (ARGs) as targets to determine resistance. Common methods include utilization of polymerase chain reaction (PCR) and its other derivative methods, including multiplex PCR (mPCR), PCR–restriction fragment length polymorphism (PCR-RFLP), quantitative PCR (qPCR), enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), and reverse transcription PCR (RT-PCR) (Algarni et al., 2022). However, discordance with the phenotypic and genotypic resistance profile is sometimes encountered. Discrepancies in the genotype-phenotype resistance profile occur when there is a mismatch between a bacterium's genetic determinants of resistance and its observed antibiotic susceptibility profile (Bonsa et al., 2023; Sutimbekova et al., 2026).

The phenotypic assessment of the resistance profile of dominant bacteria isolated from *P. vannamei* was previously conducted by Gito et al. (2026). The study revealed higher rates of resistance among dominant bacterial isolates to rifampin and ampicillin. To further elucidate the genotypic resistance profile of the dominant bacteria from whiteleg shrimp, this study aims to determine the antibiotic resistance genes (ARGs) present in the bacterial isolates. Data that reveal discordance between phenotypic and genotypic resistance in dominant MDR bacterial isolates will provide insights into the limitations of relying on a single approach for resistance detection and highlight the importance of integrating both phenotypic and molecular methods to improve disease management in aquaculture systems.

Materials and Methods

Sample collection, bacterial isolation, and antibiotic susceptibility testing: A total of 150 whiteleg shrimp samples were collected from five shrimp farms located in Iloilo Province, Philippines (Fig. 1). Sampling sites included one hatchery in Guimbal (Site 1: 10°39'24.4"N, 122°18'09.8"E) and four grow-out farms located in Dumangas (Site 2: 10°47'39.4"N, 122°41'06.5"E), Zarraga (Site 3: 10°48'35.0"N, 122°38'25.9"E), Miagao (Site 4: 10°39'22.3"N, 122°16'01.7"E), and Leganes (Site 5: 10°47'05.1"N, 122°38'23.8"E).

Both juvenile and post-larval shrimp were collected and transported under aseptic conditions to the Biology Laboratory of the University of the Philippines Visayas–Iloilo City Campus for bacteriological analysis. For juvenile shrimp, approximately 500 mg of muscle tissue was aseptically excised and transferred into sterile 1.5-mL microcentrifuge tubes. For post-larval samples, 10 individuals were pooled into a single sterile microcentrifuge tube. Sterile 0.9% saline solution was added as a diluent, and samples were homogenized using a mechanical tissue homogenizer.

Serial ten-fold dilutions of the homogenates were prepared, and 0.1 mL aliquots from the appropriate dilutions (10^{-3} to 10^{-5}) were spread-plated onto Nutrient Agar (NA) supplemented with 0.1% NaCl. Inoculated plates were incubated at 28°C for 24 h. Following incubation, morphologically distinct bacterial colonies were selected and purified through repeated streaking on fresh NA plates.

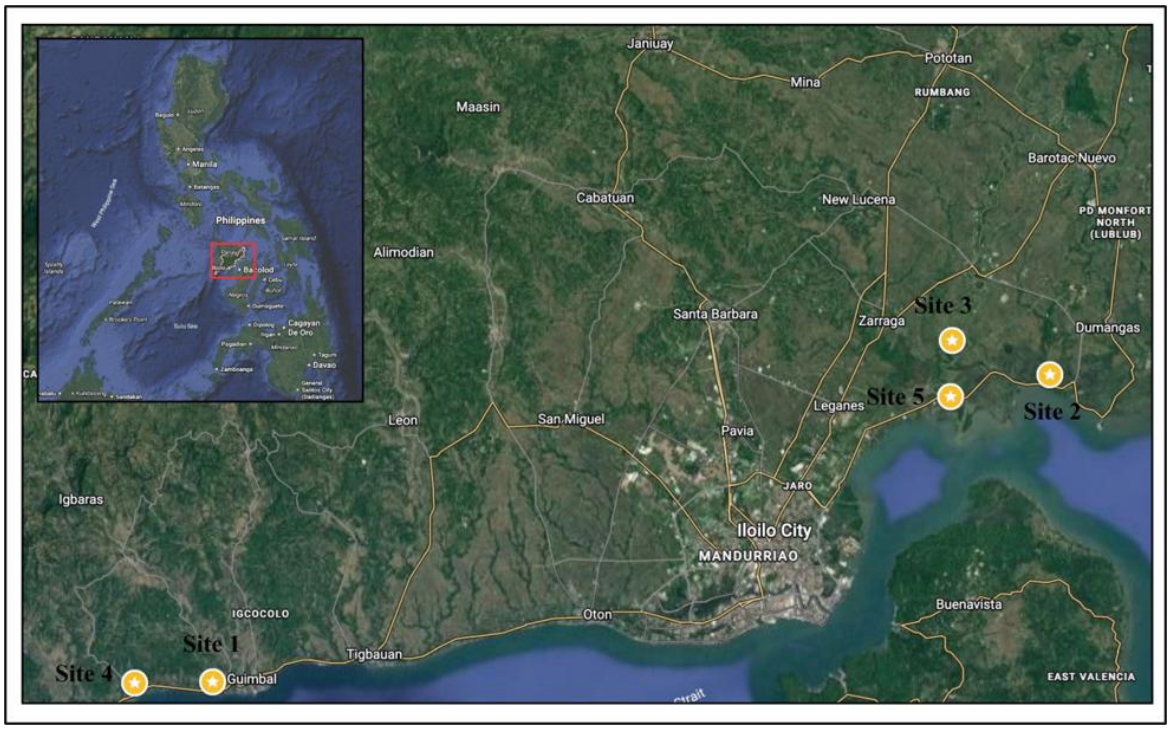

Figure 1. Map of the five sampling locations (Sites 1- 5) in the Province of Iloilo. The inset map shows the location of Iloilo Province in the Philippines. Generated in Google Maps (2026).

Preliminary screening for antibiotic-resistant bacteria was performed by culturing isolates on NA supplemented with 0.1% chloramphenicol, followed by antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method. The dominance and resistance profiles of bacterial isolates were assessed using the Antibiotic Resistance Index (ARI). Isolates with ARI values greater than 0.20 were classified as multidrug-resistant (MDR) and subsequently screened for antibiotic resistance genes (ARGs) following the protocol of Gito et al. (2026).

Genomic DNA extraction: Overnight bacterial cultures grown in Nutrient Broth supplemented with 1% NaCl were used to extract genomic DNA from dominant multidrug-resistant (MDR) isolates. Genomic DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega, USA) with minor modifications to the manufacturer's protocol. Briefly, approximately 2 mL of overnight bacterial culture was transferred into sterile 1.5-mL microcentrifuge tubes and centrifuged at 8,000 rpm for 4 min to pellet the bacterial cells. For Gram-positive isolates, the cell pellet was resuspended in 480 µL of 50 mM EDTA, then 10 µL of Proteinase K was added to facilitate cell lysis. The mixture was incubated at 37°C for 60 min and subsequently centrifuged at 8,000 rpm for 4 min. The resulting supernatant was discarded. Following the initial lysis step for Gram-positive bacteria, both Gram-positive and Gram-negative samples underwent the standard genomic DNA extraction procedure. A total of 600 µL nuclei lysis solution was added to each sample, followed by incubation at 80°C for 5 min. After cooling to room temperature, 3 µL RNase A solution was added, and the samples were incubated at 37°C for 60 min to remove RNA contaminants.

Protein precipitation was performed by adding 200 µL protein precipitation solution, followed by incubation on ice for 5 min and centrifugation at 8,000 rpm for 6 min. The resulting supernatant containing the DNA was transferred to a clean microcentrifuge tube containing 600 µL of room-temperature isopropanol and gently mixed to precipitate the DNA. Samples were centrifuged at 8,000 rpm for 9 min, after which the supernatant was carefully discarded. The DNA pellet was washed with 600 µL of chilled 70% ethanol and centrifuged at 8,000 rpm for 4 min. The ethanol was carefully aspirated, and the pellet was air-

Table 1. List of antibiotic resistance target genes, their corresponding primer sequences, amplicon sizes, and references that were used in the study.

Target Gene	Primer Sequence (5'-3')	Amplicon size (in bp)	Reference
tetA	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	210	Seyfried et al. (2010)
tetB	F: TACGTGAATTTATTGCTTCGG R: ATACAGCATCCAAAGCGCAC	206	
gyrA	F: GCGATGTCAGTTATCGTGGGT R: TTCGGTGTAACGCATTGCCGC	300	Okuda et al. (2006)
catA	F: CCAGACCGTTCAGCTGGATA R: CATCAGCACCTTGTCGCCT	454	Thaotumpitak et al. (2023)
catB	F: CGGATTCAGCCTGACCACC R: ATACGCGGTCACCTTCCTG	461	
*bla*TEM	F: ATTCTTGAAGACGAAAGGGC R: ACGCTCAGTGGAACGAAAAC	1150	Belaaouaj et al. (1994)
*bla*OXA1	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	813	Steward et al. (2001)
*erm*B	F: AGACACCTCGTCTAACCTTCGCTC R: TCCATGTACTACCATGCCACAGG	640	Raissy et al. (2012)
bla_{CTX-M}	F: ATGTGCAGYACCAGTAAGT R: TGGGTRAARTARGTSACCAGA	693	Thongkao and Sudjaroen (2020)
cat	F: GGACTGAGTGTAAGTCTGAC R: CCATACCGTTGCGT ATCAC	540	Zakaria et al. (2023)
StrA	F: TGGCAGGAGGAACAGGAGG R: AGGTCGATCAGACCCGTGC	405	Yu et al. (2023)
QnrVC	F: AATTTTAAGCGCTCAAACCTCCG R: TCCTGTTGCCACGAGCATATTTT	521	
tetC	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	418	Liu et al. (2019)
tetE	F: AAACCACATCCTCCATACGC R: AAATAGGCCACAACCGTCAG	278	
rpoB	F: GGTCGCCGCGATCAAGGAGT R: GTGCACGTCGCGGACCTCCA	159	Su et al. (2020)

dried to remove residual ethanol. Finally, the DNA pellet was rehydrated in 100 µL DNA rehydration solution and stored at 4°C overnight prior to downstream molecular analyses.

Antibiotic resistance genes detection through polymerase chain reaction (PCR): The genomic DNA extracted from dominant multidrug-resistant (MDR) bacterial isolates was screened for the presence of antibiotic resistance genes (ARGs) using polymerase chain reaction (PCR). The primer sets used to amplify the target ARGs are listed in Table 1. PCR amplification was performed in 15-µL reaction mixtures containing 4 µL BioReady 2× Taq Mix (PCR buffer, 3 mM Mg^{2+}, dNTP mixture, and Taq DNA polymerase), 6 µL sterile nuclease-free water, 1.5 µL each of forward and reverse primers, and 2 µL genomic DNA template. Amplification reactions were carried out using a GeneExplorer thermal cycler (Bioer, GE96G) under the following cycling conditions: initial denaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s; followed by a final extension at 72°C for 5 min.

PCR amplicons were analyzed by agarose gel electrophoresis. Briefly, 4 µL of each PCR product was loaded onto a 1% agarose gel stained with ethidium bromide and electrophoresed at 100 V for 30 min. The resulting DNA bands were visualized using a Gel Doc imaging system with Image Lab Software (Bio-Rad Laboratories, USA). Samples were scored as positive (+) when a distinct DNA band corresponding to the expected amplicon size of the target resistance gene was observed.

Results

A total of nine dominant bacterial isolates were

Table 2. Phenotypic and genotypic antibiotic resistance profiles of nine multidrug-resistant bacterial strains isolated in the study.

Isolate Code	Antibiotic susceptibility profile							Resistance gene profile		
	TET (30µg)	ERY (30µg)	CIP (5µg)	CHL (30µg)	RIF (5µg)	AMP (10µg)	STR (10 µg)	*tetE*	*gyrA*	*catB*
RS2	S	R	R	S	R	R	R	-	-	+
RS12	R	S	I	S	R	R	S	-	-	-
RS13	R	S	I	S	R	R	S	-	-	-
RS19	S	R	I	S	R	R	I	-	+	-
RS23	S	I	R	S	R	R	I	-	-	+
RS29	S	I	R	S	R	R	S	-	-	+
RS30	I	R	R	R	R	R	I	-	-	-
SS3	S	R	I	S	R	R	I	+	-	-
SS8	S	I	R	S	R	R	I	+	-	+

* TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; CHL, chloramphenicol; RIF, rifampin; AMP, ampicillin; STR, streptomycin.
* Resistant (R); Intermediate (I); Susceptible (S). * +, Detected; -, Not detected

Table 3. Distribution of antibiotic resistance genes detected in dominant multidrug-resistant bacterial isolates.

Antibiotic Class	**Antibiotic Resistance Gene (ARG)**	**Number of Bacterial Isolates Carrying the ARG (n)**	**ARG Carriage Rate (%)**
Chloramphenicol	*catB*	4	44.4
Tetracyclines	*tetE*	2	22.2
Quinolone	*gyrA*	1	11.1

classified as multidrug-resistant (MDR) based on resistance to at least three antibiotic classes, as determined by antibiotic susceptibility testing (Table 2). All MDR isolates (100%; 9/9) exhibited resistance to ampicillin and rifampicin. Lower proportions of resistance were observed for ciprofloxacin (55.6%; 5/9), erythromycin (44.4%; 4/9), tetracycline (22.2%; 2/9), and both chloramphenicol and streptomycin (11.1%; 1/9 each).

Screening of the MDR isolates for antibiotic resistance genes (ARGs) revealed the presence of three resistance determinants. The chloramphenicol resistance gene, *catB*, was detected in isolates RS2, RS23, RS29, and SS8. The quinolone resistance-associated gene, *gyrA*, was identified in isolate RS19, while the tetracycline resistance gene, *tetE*, was detected in isolates SS3 and SS8. Notably, isolate SS8 harbored both *catB* and *tetE* genes.

The distribution of ARGs detected among the dominant MDR bacterial isolates is summarized in Table 3. Of the 15 ARGs screened, only three genes (20%) were detected. These genes were associated with resistance to chloramphenicol (*catB*), tetracyclines (*tetE*), and quinolones (*gyrA*). Among the detected ARGs, *catB* was the most prevalent, occurring in 44.4% (4/9) of the MDR isolates. The prevalence of *tetE* and *gyrA* was 22.2% (2/9) and 11.1% (1/9), respectively. Overall, five of the nine MDR isolates (55.6%) carried at least one of the screened ARGs.

The PCR-based detection of ARGs was further validated through agarose gel electrophoresis (Fig. 2). Amplified PCR products corresponding to the *tetE*, *gyrA*, and *catB* genes were visualized and compared with their expected amplicon sizes reported in previous studies. As shown in Figure 2A-C, the observed band sizes were consistent with the predicted amplicon lengths for each target gene. The concordance between the observed and expected PCR product sizes confirms the presence of these ARGs in the dominant MDR bacterial isolates recovered from whiteleg shrimp tissues.

Discussions

The extensive and widespread use of antibiotics in aquaculture has been recognized as a major contributor to the accelerated global emergence of antibiotic resistance (Mohammed et al., 2025). The development of bacterial resistance is generally attributed to two major mechanisms: phenotypic and

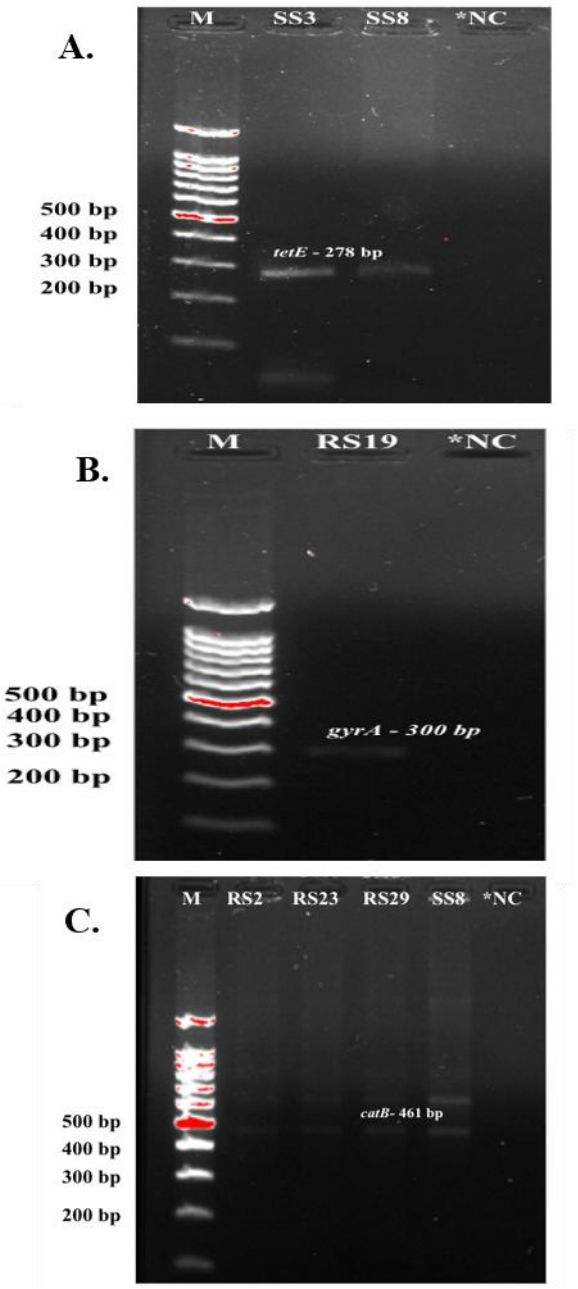

Figure 2. Gel electrophoresis visualization of antibiotic resistance genes detected in dominant multidrug-resistant bacterial isolates. Lane M is the DNA ladder (100bp). (A) Amplified PCR products visualization in gel electrophoresis for the *tetE* gene (278 bp); lanes SS3 and SS8 depict the positive samples, and lane NC depicts the negative control. (B) DNA gel electrophoresis separation of the PCR product of the *gyrA* gene (300bp); lane RS19 for positive samples of the gene and lane NC for the negative control. (C) DNA gel electrophoresis separation of the PCR product for the *catB* gene (461 bp) with positive samples in lanes *RS2, RS23, RS29, SS8,* and a negative control in lane NC.

genotypic resistance. Phenotypic resistance involves non-heritable mechanisms, including biofilm formation, cellular persistence, drug indifference, and nutrient limitation during the stationary growth phase (Corona and Martínez, 2013). In biofilm-associated resistance, the extracellular polymeric matrix produced by microorganisms acts as a physical barrier that limits antibiotic penetration into bacterial cells. Alterations in bacterial cell wall permeability may further reduce antibiotic efficacy (Pagès et al., 2008). Cellular persistence occurs when a subpopulation of bacterial cells enters a dormant state, thereby evading antibiotics that primarily target metabolically active cells. Similarly, drug indifference results from reduced antimicrobial activity against slow-growing or metabolically inactive bacteria. Nutrient limitation during the stationary phase also contributes to decreased susceptibility because many antibiotics target actively dividing cells (Corona and Martínez, 2013).

In contrast, genotypic resistance arises from genetic alterations that confer resistance traits and can be disseminated through mechanisms such as horizontal gene transfer (HGT) (Huang et al., 2022). In the present study, the molecular basis of antibiotic resistance was investigated by detecting antibiotic resistance genes (ARGs), which play a critical role in the emergence and dissemination of resistant bacterial strains (Jian et al., 2021). Although antibiotics are widely used for the prevention and treatment of bacterial infections in aquaculture, their prolonged and excessive application can lead to the accumulation of antibiotic residues and the selection of resistant bacterial populations carrying ARGs. Beyond their persistence in the environment, ARGs pose a significant threat because they can be horizontally transferred among bacteria. Resistance genes located on mobile genetic elements such as integrons, transposons, and plasmids may be exchanged between bacteria of the same or different species through conjugation, transformation, and transduction (Dantas et al., 2008; Yuan et al., 2023).

In this study, discrepancies between the phenotypic and genotypic resistance profiles of the MDR bacterial isolates were observed. Several isolates exhibited phenotypic resistance to specific antibiotics despite the absence of the corresponding resistance genes

among those screened. Such inconsistencies may be attributed to the presence of alternative resistance genes not included in the screening panel or to other mechanisms conferring resistance to antibiotics such as rifampicin and ampicillin. Similar discrepancies between phenotypic and genotypic resistance profiles have been reported in previous studies (Deng et al., 2016; Nadella et al., 2024).

Although bacterial resistance can be evaluated using both phenotypic and genotypic approaches, the results obtained from these methods do not always correspond. Resistance genes may be present but remain unexpressed during phenotypic testing, resulting in apparent susceptibility despite the genetic potential for resistance. This phenomenon is commonly associated with gene silencing, wherein the expression of resistance genes is reduced or completely suppressed (Rasheed et al., 2023). Such genes are often referred to as silent or cryptic genes, which remain unexpressed or minimally expressed under standard laboratory conditions. Their activation may occur through mutations, insertions, recombination events, or other genetic modifications (Stasiak et al., 2021).

Several mechanisms may contribute to the lack of expression of resistance genes. One major mechanism involves mutations that alter the DNA sequence and disrupt gene function. Such alterations may result in frameshift, missense, or nonsense mutations, as well as the loss of translation initiation elements, the separation of the promoter from coding sequences, or the truncation of regulatory regions. One well-documented example is the phenomenon known as Silencing of Antibiotic Resistance by Mutation (SARM), in which mutations occurring upstream of resistance genes suppress their expression. This mechanism has been reported in *Staphylococcus aureus* strains carrying resistance genes that remain phenotypically silent. In a study involving 1,470 *S. aureus* isolates, approximately 10% carried silent resistance genes, with 3.1% exhibiting silencing through the SARM mechanism (Wong et al., 2009; Kime et al., 2019; Deekshit and Srikumar, 2022).

Another possible explanation for the non-expression of resistance genes is the disruption of regulatory pathways controlling gene expression. Antibiotic resistance genes are regulated by both positive and negative regulatory elements, and silencing may result from defective promoters or the action of strong repressor proteins (Sánchez and Demain, 2015). This mechanism has been demonstrated in certain *S. aureus* strains that carry the *mecA* gene yet remain susceptible to oxacillin. In such cases, the regulatory proteins MecI and MecR1 suppress *mecA* expression, thereby preventing phenotypic resistance (Safo et al., 2006; Pournaras et al., 2013).

Integrons may also influence the expression of resistance genes. These mobile genetic elements contain gene cassettes that can position resistance genes at varying distances from promoter regions. Gene cassettes located closer to promoter sequences tend to exhibit higher expression levels, whereas those positioned farther away may show reduced transcriptional activity, resulting in lower phenotypic expression of resistance (Carattoli, 2001).

Another mechanism that may suppress resistance gene expression is xenogeneic silencing, a bacterial defense strategy that represses foreign DNA acquired through horizontal gene transfer. In this process, specialized proteins bind to and silence foreign genetic elements, including ARGs. Examples of xenogeneic silencers include H-NS in Gram-negative Proteobacteria, MvaT and MvaU in *Pseudomonas* spp., Lsr2 in Actinobacteria, and Rok in *Bacillus subtilis* (Ali et al., 2012).

In addition to biological factors, laboratory conditions may also contribute to discrepancies between phenotypic and genotypic resistance profiles. Variables such as culture media composition, incubation conditions, and bacterial handling procedures may influence the expression of resistance genes during phenotypic testing (Koskiniemi et al., 2011). Likewise, technical factors associated with PCR-based detection, including primer design, amplification efficiency, and primer GC content, may affect the successful detection of ARGs during genotypic analysis (Anjum et al., 2017).

The ARGs detected in the dominant MDR bacterial isolates in this study conferred resistance to chloramphenicol, tetracyclines, and quinolones. Similar ARGs have previously been reported in shrimp tissues and aquaculture environments. Liu et al. (2019) detected a high prevalence of tetracycline resistance genes, including *tetA*, *tetB*, *tetC*, and *tetE*, in shrimp gut microbiota using both culture-dependent and culture-independent PCR-based approaches. The quinolone resistance gene *qnrS* was also identified. Likewise, quinolone resistance genes, such as qnrVC, have been reported in shrimp culture environments (Yu et al., 2023), while chloramphenicol resistance genes, such as *cmlA*, have been detected in aquaculture-associated bacterial populations (Xue et al., 2021).

Chloramphenicol is a broad-spectrum bacteriostatic antibiotic that exerts its antimicrobial activity by binding to the 50S ribosomal subunit and inhibiting peptidyl transferase activity, thereby preventing protein synthesis. Resistance is commonly mediated by chloramphenicol acetyltransferases, which enzymatically inactivate the antibiotic through acetylation (Schmitz and Fluit, 2010). For example, *Vibrio harveyi* possesses the *cat2* gene, which contributes to chloramphenicol resistance by inactivating the antibiotic. Furthermore, the *cfr* gene, encoding a 23S rRNA methyltransferase, confers resistance by modifying the antibiotic-binding site on the ribosome (Yu et al., 2023; Fukuda et al., 2024).

The tetracycline resistance gene *tetE* was also detected in the present study. Tetracycline resistance genes are commonly reported in aquaculture-associated bacteria, including *Aeromonas hydrophila*. Genes such as *tetA*, *tetC*, *tetE*, and *tetM* confer resistance through two principal mechanisms: ribosomal protection proteins that prevent tetracycline from binding to ribosomes and efflux pumps that actively export the antibiotic from bacterial cells (Defoirdt et al., 2011; Li et al., 2024; Akter et al., 2025).

Quinolone resistance genes were likewise detected among the MDR isolates. Resistance to quinolones may arise through chromosomal mutations that affect target enzymes, reduced intracellular accumulation of the antibiotic, or plasmid-mediated mechanisms (Solano-Gálvez et al., 2021). In aquaculture environments, *Aeromonas* and *Vibrio* species have been reported to carry genes such as *qnrVC*, *oqxB*, and *soxR*, which confer resistance by protecting DNA gyrase and topoisomerase IV from quinolone inhibition, thereby preserving bacterial DNA replication processes (Defoirdt et al., 2011; Yu et al., 2023).

Conclusion

This study demonstrated the presence of multidrug-resistant (MDR) bacteria in cultured whiteleg shrimp collected from shrimp farms and a hatchery in Iloilo Province, Philippines, indicating antibiotic resistance in local aquaculture systems. Phenotypic characterization of the dominant MDR bacterial isolates revealed complete resistance to ampicillin and rifampicin, with lower resistance frequencies to erythromycin, tetracycline, chloramphenicol, and streptomycin. Genotypic analysis by polymerase chain reaction (PCR) confirmed the presence of antibiotic resistance genes conferring chloramphenicol, tetracycline, and quinolone resistance, with the *catB* gene being the most prevalent ARG in the MDR isolates. Notably, discrepancies were observed between the phenotypic and genotypic resistance profiles of several MDR isolates, as resistance phenotypes were not always accompanied by the detection of the corresponding antibiotic resistance genes. These findings suggest that additional determinants of resistance or alternative mechanisms, including gene regulation, gene silencing, physiological adaptations, and other uncharacterized resistance pathways, may contribute to the observed antimicrobial resistance. Overall, the study highlights the occurrence of antibiotic-resistant bacteria and antibiotic resistance genes in cultured *P. vannamei*, emphasizing the need for continuous antimicrobial resistance surveillance and prudent antibiotic use in aquaculture production systems.

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