Antimicrobial resistance genes and salt-dependent antibiotic susceptibility in *Vibrio alginolyticus* isolated from diseased *Sciaenops ocellatus* (Linnaeus, 1766) in Vietnam

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Abstract. This study aimed to molecularly characterize antimicrobial resistance (AMR) genes and evaluate the influence of salinity on antibiotic susceptibility in *Vibrio alginolyticus* isolated from diseased red drum, *Sciaenops ocellatus* (Linnaeus, 1766), in Central Vietnam. The isolates were identified by species-specific polymerase chain reaction (PCR) and screened for resistance-associated genes using PCR-based assays. Five isolates were tested for susceptibility to 21 antibiotics across seven NaCl concentrations (0-4%) using the disk diffusion method. The isolates harbored multiple AMR genes, including those for aminoglycosides (*strA*, *strB*), β-lactams (*bla*$_{\text{SHV}}$, *bla* $_{\text{CTX-M}}$), tetracyclines (*tetB*, *tetE*, *tetG*), fluoroquinolones (*qnrA*, *qnrB*, *qnrS*), sulfonamides (*sul1*, *sul2*, *sul3*), macrolides (*ermA*, *ermB*, *ermC*), and individual determinants for phenicol (*fexA*), polymyxin (*mcr*), and multidrug resistance (*cfr*). Phenotypically, the isolates were resistant to amoxicillin, ampicillin, vancomycin, streptomycin, and trimethoprim/sulfamethoxazole. Increasing NaCl concentrations enhanced resistance, particularly to fluoroquinolones and tetracyclines. At 3-4% NaCl, inhibition zones for ciprofloxacin and doxycycline decreased by up to 40% compared to standard conditions. One representative isolate (VA01) was selected for PCR-based screening, revealing a diverse resistance gene reservoir associated with *S. ocellatus* vibriosis in Vietnam. These findings highlight the importance of integrating molecular surveillance and salinity-aware antibiotic susceptibility testing to support effective antimicrobial management and disease control strategies in marine aquaculture.

Key Words: *Vibrio* species, antibiotic susceptibility, AMR genes, marine aquaculture, fish pathogen.

Introduction. Antibiotics have been widely used to control bacterial infections in aquaculture, particularly in intensive production systems. Between 2008 and 2018, approximately 44.77 million tons of antibiotics were applied in aquaculture across Asia, with China, Bangladesh, and Vietnam identified as regions with the highest usage (Lulijwa et al 2020). The extensive, often prophylactic, use of antibiotics has raised serious concerns about the emergence and spread of antibiotic-resistant bacteria in aquatic environments.

In Vietnam, antibiotic use in aquaculture remains widespread and is often administered without prior disease diagnosis or antibiotic susceptibility testing. Surveys have shown that 91.7% of farmers in the Red River Delta used antibiotics for disease treatment, of whom 98.2% did not assess antibiotic susceptibility before use, and 78.9% administered antibiotics immediately upon observing fish mortalities (Dang et al 2021). Similarly, high levels of antibiotic use have been reported among tilapia and pangasius farmers in the Mekong Delta, often involving frequent switching between drugs when treatments fail (Ström et al 2019). A nationwide survey across seven provinces further confirmed that antibiotics, mainly phenicols, tetracyclines, and sulfonamides, were used in 64% of fish farms and 24% of shrimp farms (Luu et al 2021).

Vibrio alginolyticus is a Gram-negative, motile bacterium ubiquitously distributed in estuarine and coastal environments. Together with other pathogenic *Vibrio* species, including *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus*, it represents a major threat to aquatic animal health and aquaculture production worldwide (Baker-Austin et al 2018). The indiscriminate and prolonged use of antibiotics for bacterial disease control has accelerated the emergence of antibiotic-resistant *Vibrio* strains, contributing not only to disease management challenges but also to environmental contamination and potential public health risks (Caputo et al 2023). In Vietnam, *V. alginolyticus* and other *Vibrio* species isolated from aquaculture environments have shown high resistance to commonly used antibiotics, including β-lactams, aminoglycosides, and tetracyclines (Ngo et al 2022; Nguyen et al 2024).

Despite increasing evidence of AMR in marine *Vibrio* species, the effects of environmental factors, particularly salinity, on resistance expression remain poorly understood. Salinity is a key ecological driver in marine environments and may affect bacterial physiology and phenotypic antibiotic susceptibility. Therefore, this study aimed to identify AMR genes and examine the influence of salinity on phenotypic resistance in a *V. alginolyticus* strain isolated from diseased red drum, *Sciaenops ocellatus* (Linnaeus, 1766) in Central Vietnam. This line of research contributes baseline information on the adaptive responses of *V. alginolyticus* under saline conditions and is relevant to the development of antimicrobial stewardship strategies within the "One Health" framework, which recognizes the interconnectedness of human, animal, and environmental health in addressing AMR (WHO 2015).

Material and method

Bacterial strain. Five gram-negative isolates, designated VA01-VA05, were isolated in March 2023 from diseased *S. ocellatus* showing hemorrhagic lesions in Hue City, Central Vietnam. Tissue samples (head kidney) were aseptically excised and streaked onto thiosulfate-citrate-bile salts-sucrose (TCBS, Hi Media, India) agar supplemented with 2% NaCl, followed by incubation at 28°C for 24 h. Presumptive *Vibrio* colonies were purified by repeated streaking on tryptone soy agar supplemented with 2% NaCl.

Biochemical characteristics were determined using the API 20E system (bioMérieux, France), and species identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF) as *V. alginolyticus* at the University of Sydney (Australia).

Effect of NaCl on antibiotic resistance phenotype. The influence of NaCl concentration on antibiotic resistance was assessed using the disk diffusion method on Mueller-Hinton agar (MHA; HiMedia, India), following the procedure of Uwizeyimana et al (2020). Sodium chloride was incorporated directly into the MHA medium at final concentrations of 0-4%, while antibiotic discs were used without modification.

Antibiotics were selected based on: (i) commonly authorized antibiotics for aquaculture in Vietnam (MARD 2016); (ii) those frequently detected as residues in aquatic products (MARD 2022); and (iii) antibiotics of clinical relevance in human and veterinary medicine. This selection allowed for a comprehensive assessment of *V. alginolyticus* resistance across multiple antibiotic classes. A total of 21 antibiotics representing nine classes were tested (Table 1).

Each isolate was cultured in 10 mL of tryptone soy broth (TSB; HiMedia, India) supplemented with 2% NaCl at 28°C and 150 rpm for 24 h. The optical density at 600 nm (OD_{600}) was adjusted to 1.0 (equivalent to 10^9 CFU mL^{-1}) and diluted to 10^6 CFU mL^{-1}. One hundred microliters of the suspension were spread on MHA plates containing 0-4% NaCl (0%, 0.5%, 1.0%, 1.5%, 2.0%, 3.0%, and 4.0%). All assays were performed in triplicate for each isolate, antibiotic, and NaCl concentration. After air-drying at 28°C for 30 min, antibiotic discs (Nam Khoa Co., Vietnam) were placed on the plates, which were then incubated at 28°C for 24 h.

Inhibition zone diameters were measured and interpreted following CLSI (2015) and CLSI (2024).

Table 1

List of antibiotics tested and their classification

No.	*Groups*	*Antibiotic*	*Symbol*	*Disk content (µg)*
1	Betalactam	Amoxicillin	Ax	10
2		Ampicillin	Am	10
3		Cephalecin	Cp	30
4		Cefotaxim	Ct	30
5	Aminoglycoside	Streptomycin	Sm	10
6		Gentamycin	Ge	10
7		Neomycin	Ne	30
8		Kanamycin	Kn	30
9	Glycopeptides	Vancomycin	Va	30
10	Macrolides	Erythromycin	Er	15
11		Clarithromycin	Ch	15
12		Azithromycin	Az	15
13	Fluoroquinolones	Enrofloxacin	Ef	5
14		Ofloxacin	Of	5
15		Ciprofloxacin	Ci	5
16		Norfloxacin	Nr	10
17	Sulfonamide	Trimethoprim/Sulfamethoxazole	Bt	1.25/23.75
18	Phenicol	Florfenicol	Fl	30
19	Tetracycline	Doxycycline	Dx	30
20		Tetracycline	Te	30
21	Rifamycin	Rifampicin	Rf	5

Note: Twenty-one antibiotics representing nine major classes were used to assess the susceptibility of *Vibrio alginolyticus* isolates under varying NaCl concentrations (0-4%). Antibiotics were selected based on their relevance in aquaculture, veterinary, and human medicine. Concentrations and disc potencies follow CLSI (M45, 2015; M100, 2024) guidelines.

Selection of a representative isolate for genotyping. All five *V. alginolyticus* isolates were initially subjected to antibiotic susceptibility testing under varying NaCl concentrations. One representative isolate (VA01) was selected for genetic analysis based on its phenotypic resistance profile being representative of the majority of isolates, consistent salt-dependent variation in inhibition zone diameters, and stable growth and virulence characteristics observed during preliminary assays.

Detection of antibiotic resistance genes. Twenty-one AMR genes representing β-lactam, aminoglycoside, macrolide, tetracycline, fluoroquinolone, sulfonamide, phenicol, polymyxin, and multidrug-resistance classes were screened by PCR (Table 2).

Genomic DNA of *V. alginolyticus* strains was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Briefly, bacterial cells were pelleted by centrifugation (Eppendorf 5424R, 12,000 rpm, 2 min) and lysed with Buffer ATL and Proteinase K at 56°C for 30 min (Eppendorf ThermoMixer C). DNA was then purified using silica membrane spin columns and eluted with 50 µL Buffer AE. DNA concentration and purity were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA).

PCR amplification was carried out in a Bio-Rad T100™ Thermal Cycler (USA) using 2× PCR Master Mix (commercial supplier), specific primer pairs, and the extracted DNA as template. The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing temperatures listed in Table 2 for 30 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 5 min. PCR products were resolved by electrophoresis on 2% agarose gels in 1× TAE buffer at 100 V for 20 min, stained with GelRed, and visualized using the runVIEW™ system. The GeneRuler™ 100 bp Plus DNA Ladder (Thermo Scientific, USA) was used as a molecular size marker. Positive and negative controls were included in all reactions. PCR products were visualized on 1.5% agarose gels stained with ethidium bromide and documented using a UV transilluminator.

Table 2

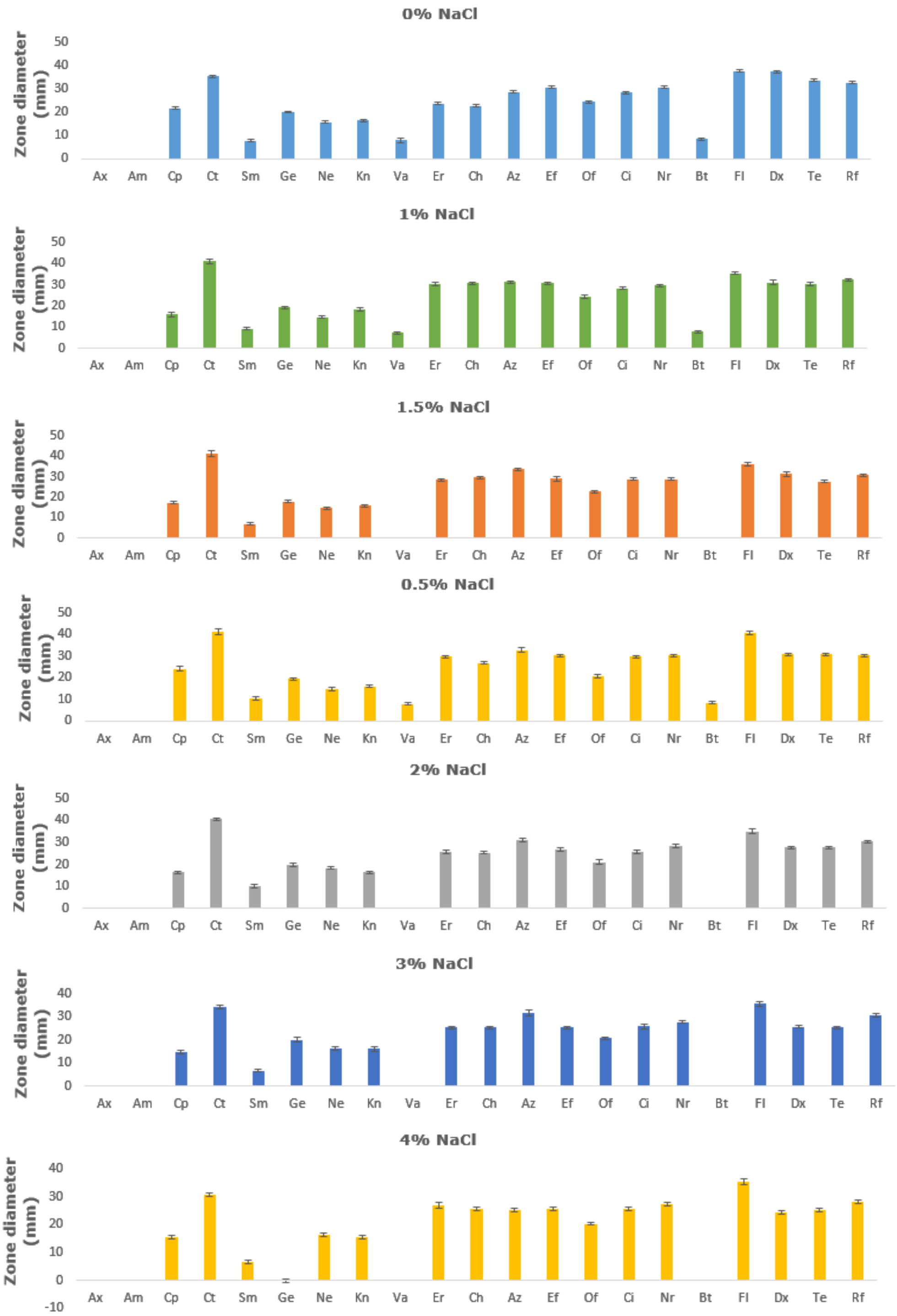
Primers sequences used for the amplification of antimicrobial resistance genes

No.	*Targeted gene*	*Primer name*	*Sequence (5′ - 3′)*	*Product size (bp)*	*References*
1	*qnrA*	*qnrA*-F *qnrA*-R	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	580	Costa et al 2021
2	*qnrB*	*qnrB*-F *qnrB*-R	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	264	
3	*qnrS*	*qnrS*-F *qnrS*-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	
4	bla_{SHV}	bla_{SHV}-F bla_{SHV}-R	CTTGACCGCTGGGAAACGG AGCACGGAGCGGATCAACGG	200	
5	*ermA*	*ermA-F* *ermA-R*	TATCTTATCGTTGAGAAGGGATT CTACACTTGGCTTAGGATGAAA	139	Mahdi Al-Buhilal et al 2021
6	*ermB*	*ermB-F* *ermB-R*	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	
7	*ermC*	*ermC-F* *ermC-R*	CTTGTTGATCACGATAATTTCC ATCTTTTAGCAAACCCGTATTC	190	
8	*tetB*	*tetB*-F *tetB*-R	ACGTTACTCGATGCCAT AGCACTTGTCTCCTGTT	1169	Letchumanan et al 2015
9	*tetG*	*tetG*-F *tetG*-R	CCGGTCTTATGGGTGCTCTA CCAGAAGAACGAAGCCAGTC	603	
10	bla_{OXA}	bla_{OXA}-F bla_{OXA}-R	ACCAGATTCAACTTTCAA TCTTGGCTTTTATGCTTG	589	
11	*tetE*	*tetE*-F *tetE*-R	GTTATTACGGGAGTTTGTTGG AATACAACACCCACACTACGC	199	Aminov et al 2002; Hoa et al 2008
12	*sul1*	*sul1*-F *sul1*-R	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433	
13	*sul2*	sul2-F sul2-R	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT	293	
14	*sul3*	pVP440sul3F pVP440sul3R	TCAAAGCAAAATGATATGAGC TTTCAAGGCATCTGATAAAGAC	787	
15	*armA*	*armA*-F *armA*-R	AGGTTGTTTCCATTTCTGAG TCTCTTCCATTCCCTTCTCC	590	Yamane et al 2005
16	*aac(3)-IIa*	*aac(3)-IIa*-F *aac(3)-IIa*-R	CGGAAGGCAATAACGGAG TCGAACAGGTAGCACTGAG	740	Maynard et al 2004
17	bla_{CTX-M}	bla_{CTX-M}-F bla_{CTX-M}-R	AATCACTGCGTCAGTTCAC TTTATCCCCCACAACCCAG	701	
18	*strA-strB*	*strA-strB*-F *strA-strB*-R	GGTTGCCTGTCAGAGGCGG GTCAGAGGGTCCAATCGC	750	Sundin et al 2002
19	*cfr*	*cfr-F* *cfr-R*	TGAAGTATAAAGCAGGTTGGGAGTCA ACCATATAATTGACCACAAGCAGC	746	Kehrenberg & Schwarz, 2006
20	*fexA*	*fexA-F* *fexA-R*	GTACTTGTAGGTGCAATTACGGCTGA CGCATCTGAGTAGGACATAGCGTC	1272	
21	mcr_1	MCR_1-F MCR_1-R	GTCGGTATGCTCGTTGGCTTAG CATAGGCATTGCTGTGCGTCTG	715	Rebelo et al 2018

Statistical analysis. All experiments were conducted in triplicate, and data are expressed as the mean ± standard deviation (SD) unless otherwise specified. Before analysis, data were tested for normality using the Shapiro-Wilk test. Presence or absence of AMR genes was recorded qualitatively, while quantitative data (e.g., inhibition zone diameters under different NaCl concentrations) were statistically analyzed using IBM SPSS Statistics 20 (IBM Corp., USA). One-way analysis of variance (ANOVA) was applied to evaluate the effects of NaCl concentration on antibiotic resistance and other quantitative parameters, followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Statistical significance was accepted at $p < 0.05$.

Results

***Antibiotic resistance profiles under different NaCl concentrations*.** All five *V. alginolyticus* isolates exhibited similar resistance patterns across NaCl concentrations ranging from 0-4%. Under all tested salinity conditions, 100% of the isolates were resistant to amoxicillin, ampicillin, vancomycin, streptomycin, and trimethoprim/sulfamethoxazole (Figure 1).


0% NaCl

1% NaCl

1.5% NaCl

0.5% NaCl

2% NaCl

3% NaCl

4% NaCl

Figure 1. Antibiotic resistance profiles of five *Vibrio alginolyticus* isolates under different NaCl concentrations (0-4%). Data are presented as mean inhibition zone diameters ± standard deviation (n = 3). Different lowercase letters within the same antibiotic indicate statistically significant differences among NaCl concentrations ($p < 0.05$, one-way ANOVA followed by Tukey's HSD test).

Across the five isolates, increasing NaCl concentrations consistently resulted in reduced inhibition zone diameters for most antibiotics, indicating a common salinity-dependent trend toward enhanced phenotypic resistance (Figure 1). Because this pattern was consistently observed in all isolates, one representative isolate (VA01) was selected for detailed quantitative analysis of the relationship between NaCl concentration and antibiotic susceptibility

In isolate VA01, inhibition zone diameters for fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin) and tetracyclines (tetracycline, doxycycline) showed a clear salinity-dependent decline. At high salinity levels (3-4% NaCl), inhibition zones diameters for ciprofloxacin and doxycycline decreased by approximately 30-40% compared with those observed under standard conditions (0-0.5% NaCl) (Figure 2).

Conversely, moderate NaCl levels (0.5-2%) resulted in a slight increase in susceptibility to certain antibiotics, including the β-lactam cefotaxime and macrolides (erythromycin, clarithromycin), as reflected by marginally larger inhibition zones (Figure 1).

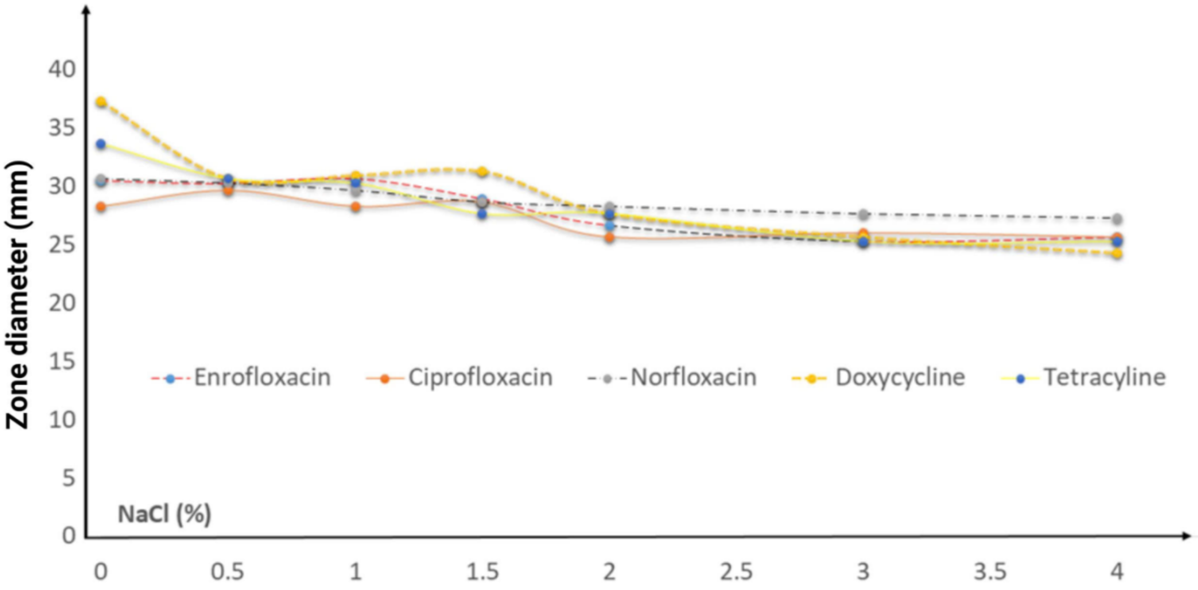


Figure 2. Effect of NaCl concentration on inhibition zone diameters of selected antibiotics in *Vibrio alginolyticus* isolate VA01. Values represent mean ± standard deviation (n = 3). Increasing salinity was associated with a progressive reduction in inhibition zone diameters for fluoroquinolones and tetracyclines.

Detection of antimicrobial resistance genes in representative isolate VA01. PCR screening of the representative isolate VA01 revealed the presence of a broad spectrum of AMR genes (Table 3). Of the 21 target genes examined, 18 genes (85.7%) were detected. These included genes associated with resistance to fluoroquinolones (*qnrA*, *qnrB*, *qnrS*), β-lactams (*blaSHV*, *blaCTX-M*), macrolides (*ermA*, *ermB*, *ermC*), tetracyclines (*tetB*, *tetE*, *tetG*), sulfonamides (*sul1*, *sul2*, *sul3*), aminoglycosides (*strA*-*strB*), phenicols (*fexA*), polymyxins (*mcr*), and multidrug resistance (*cfr*).

In contrast, three resistance genes (*blaOXA*, *armA*, and *aac*(3)-IIa) were not detected in isolate VA01. The coexistence of multiple resistance genes was consistent with the multidrug-resistant phenotypes observed under different salinity conditions.

Table 3

PCR detection results of antibiotic resistance genes in the *Vibrio alginolyticus* strains

No.	*Targeted gene*	*Primer name*	*Sequence (5′ - 3′)*	*PCR*	*AMR for*
1	*qnrA*	*qnrA*-F	AGAGGATTTCTCACGCCAGG	+	Fluoroquinolones
		qnrA-R	TGCCAGGCACAGATCTTGAC		
2	*qnrB*	*qnrB*-F	GGMATHGAAATTCGCCACTG	+	
		qnrB-R	TTTGCYGYYCGCCAGTCGAA		
3	*qnrS*	*qnrS*-F	GCAAGTTCATTGAACAGGGT	+	
		qnrS-R	TCTAAACCGTCGAGTTCGGCG		
4	*bla*$_{SHV}$	*bla*$_{SHV}$-F	CTTGACCGCTGGGAAACGG	+	Betalactam
		bla$_{SHV}$-R	AGCACGGAGCGGATCAACGG		
5	*bla*$_{CTX-M}$	*bla*$_{CTX-M}$-F	AATCACTGCGTCAGTTCAC	+	
		bla$_{CTX-M}$-R	TTTATCCCCCACAACCCAG		
6	*bla*$_{OXA}$	*bla*$_{OXA}$-F	ACCAGATTCAACTTTCAA	-	
		bla$_{OXA}$-R	TCTTGGCTTTTATGCTTG		
7	*ermA*	*ermA-F*	TATCTTATCGTTGAGAAGGGATT	+	Macrolides
		ermA-R	CTACACTTGGCTTAGGATGAAA		
8	*ermB*	*ermB-F*	CTATCTGATTGTTGAAGAAGGATT	+	
		ermB-R	GTTTACTCTTGGTTTAGGATGAAA		
9	*ermC*	*ermC-F*	CTTGTTGATCACGATAATTTCC	+	
		ermC-R	ATCTTTTAGCAAACCCGTATTC		
10	*tetB*	*tetB*-F	ACGTTACTCGATGCCAT	+	Tetracyline
		tetB-R	AGCACTTGTCTCCTGTT		
11	*tetE*	*tetE*-F	GTTATTACGGGAGTTTGTTGG	+	
		tetE-R	AATACAACACCCACACTACGC		
12	*tetG*	*tetG*-F	CCGGTCTTATGGGTGCTCTA	+	
		tetG-R	CCAGAAGAACGAAGCCAGTC		
13	*sul1*	*sul1*-F	CGGCGTGGGCTACCTGAACG	+	Sulfonamides
		sul1-R	GCCGATCGCGTGAAGTTCCG		
14	*sul2*	sul2-F	GCGCTCAAGGCAGATGGCATT	+	
		sul2-R	GCGTTTGATACCGGCACCCGT		
15	*sul3*	pVP440sul3F	TCAAAGCAAAATGATATGAGC	+	
		pVP440sul3R	TTTCAAGGCATCTGATAAAGAC		
16	*armA*	*armA*-F	AGGTTGTTTCCATTTCTGAG	_	Aminoglycoside
		armA-R	TCTCTTCCATTCCCTTCTCC		
17	*aac(3)-IIa*	*aac(3)-IIa*-F	CGGAAGGCAATAACGGAG	_	
		aac(3)-IIa-R	TCGAACAGGTAGCACTGAG		
18	*strA-strB*	*strA-strB*-F	GGTTGCCTGTCAGAGGCGG	+	
		strA-strB-R	GTCAGAGGGTCCAATCGC		
19	*fexA*	*fexA-F*	GTACTTGTAGGTGCAATTACGGCTGA	+	Phenicol
		fexA-R	CGCATCTGAGTAGGACATAGCGTC		
20	*mcr*$_1$	*MCR*$_1$*-F*	GTCGGTATGCTCGTTGGCTTAG	+	Polymyxin
		MCR$_1$*-R*	CATAGGCATTGCTGTGCGTCTG		
21	*cfr*	*cfr-F*	TGAAGTATAAAGCAGGTTGGGAGTCA	+	MDR
		cfr-R	ACCATATAATTGACCACAAGCAGC		

Note: "+" indicates a positive PCR result, and "−" indicates a negative PCR result.

Relationship between resistance genotypes and phenotypes. Although multiple resistance genes were detected, phenotypic resistance levels varied depending on NaCl concentration. This observation suggests that environmental salinity may modulate the phenotypic expression of resistance, potentially through physiological or regulatory mechanisms rather than gene presence alone.

Discussion

Salinity-dependent modulation of antibiotic susceptibility in Vibrio alginolyticus. Salinity markedly influenced antibiotic susceptibility in the tested *V. alginolyticus* strains. At low NaCl concentrations (0-1.0%), incomplete resistance to vancomycin, streptomycin, and trimethoprim/sulfamethoxazole was observed, whereas at higher salinities (1.5-4.0%), inhibition zones disappeared, indicating enhanced bacterial tolerance. A similar salinity-dependent reduction in inhibition zones was observed for fluoroquinolones (enrofloxacin, ciprofloxacin, norfloxacin) and tetracyclines (doxycycline, tetracycline).
Although a consistent salinity-dependent trend was evident for fluoroquinolones and tetracyclines, the influence of NaCl on resistance to other antibiotics was less uniform. Such variability may reflect differences among antibiotic classes and experimental conditions that influence bacterial susceptibility, as previously reported (Ottaviani et al 2001). Overall, these findings support the view that elevated salinity can reduce antibiotic effectiveness and promote phenotypic resistance in marine *Vibrio* strains

Physiological mechanisms underlying salinity-mediated antibiotic tolerance. Enhanced bacterial growth under high-salinity conditions is likely associated with physiological and metabolic adjustments in response to osmotic stress. Increased NaCl concentrations have been reported to stimulate the pyruvate cycle, resulting in elevated ATP production that may fuel efflux pump activity and facilitate antibiotic expulsion (Yang et al 2018). Comparable salt-induced tolerance mechanisms have been described in *Salmonella* (Yoon et al 2013) and *Escherichia coli* (Zhu & Dai 2018), suggesting that salinity-mediated antibiotic tolerance may represent a conserved bacterial stress response.

In marine *Vibrio* species, salinity stress may further alter membrane permeability, cellular redox balance, and energy metabolism, collectively contributing to reduced antibiotic susceptibility (Gregory & Boyd 2021). Consistent with this interpretation, transcriptomic studies in *V. parahaemolyticus* have demonstrated that salinity fluctuations modulate the expression of genes associated with transport systems, redox metabolism, and multidrug efflux pumps such as acrAB-tolC and norM (Ma et al 2021). In addition, genomic adaptations enhancing metabolic flexibility and stress resilience under high-salinity conditions have been reported in *Vibrio campbellii*, indicating that salinity may act as a trigger for stress-response pathways linked to antibiotic tolerance (Pattano et al 2025).

Resistance patterns in the context of aquaculture practices in Vietnam. The *V. alginolyticus* strains isolated from *S. ocellatus* cultured in Central Vietnam exhibited resistance to amoxicillin, ampicillin, vancomycin, streptomycin, and trimethoprim/sulfamethoxazole. Among these antibiotics, vancomycin is prohibited for use in aquaculture, whereas ampicillin is rarely applied due to its instability in aquatic environments and widespread resistance. In contrast, amoxicillin, streptomycin, and trimethoprim/sulfamethoxazole remain commonly used for bacterial disease control in aquaculture systems (MARD 2016).

Resistance to amoxicillin and streptomycin has frequently been reported in *V. alginolyticus* isolates from northern Vietnam, while ampicillin resistance has been documented in strains from both northern and southern regions (Ngo et al 2022; Nguyen et al 2024). These observations indicate that the resistance patterns detected in the present study are consistent with broader national trends.

Relationship between resistance genotypes and phenotypic expression under salinity stress. Amoxicillin and ampicillin, both belonging to the penicillin subclass of β-lactam antibiotics, are commonly inactivated by bacteria carrying the *bla*$_{SHV}$ gene (Nakayama et al 2023). In this study, all five *V. alginolyticus* isolates harbored *bla*$_{SHV}$ and exhibited complete resistance across all tested salinity conditions, supporting the functional relevance of this gene in β-lactam resistance.

Resistance to aminoglycosides is typically mediated by efflux systems or enzymatic modification. The detection of *strA-strB* in isolate VA01, in the absence of *armA* and *aac*(3)-*IIa*, was consistent with the observed phenotypic resistance to streptomycin. Similar resistance mechanisms involving *strA-strB* have been widely reported in aquatic bacteria (Jassem et al 2014; Vaiyapuri et al 2021).

Sulfonamide resistance is commonly mediated by *sul1, sul2,* and *sul3,* which have been reported in *V. alginolyticus* isolated from marine fish, coastal aquaculture systems, seafood, and freshwater aquaculture environments (Hoa et al 2008; Mohamad et al 2019; Yu et al 2022; Sun et al 2024). In this study, all three *sul* genes were detected and corresponded with phenotypic resistance to trimethoprim/sulfamethoxazole, consistent with previous reports indicating that *sul1* and *sul2* are generally more prevalent than *sul3* (Huang et al 2024).

Fluoroquinolone resistance is mediated by *qnrA, qnrB,* and *qnrS* (Xu et al 2023), whereas tetracycline resistance is often associated with efflux pumps encoded by *tetB, tetE,* and *tetG*. These genes have been widely reported in *V. alginolyticus* and other *Vibrio* species from aquaculture environments (Yu et al 2022; Minimol et al 2024; Algammal et al 2025). Although these resistance genes were detected in the representative isolate, phenotypic resistance was not observed under standard salinity conditions. In this context, gene silencing refers to the presence of resistance genes that are not actively expressed or are expressed at levels insufficient to confer phenotypic resistance under the tested conditions. Such genes may become functionally relevant under favorable environmental conditions or through mutation, recombination, or horizontal gene transfer (Stasiak et al 2021).

Broader implications and study limitations. The detection of additional resistance determinants, including *ermA*, *ermB*, and *ermC* (macrolides), *fexA* (phenicol), *cfr*(multidrug resistance), and *mcr* (polymyxin resistance), highlights the potential risk of AMR dissemination in aquaculture systems (Kehrenberg et al 2006; Yin et al 2022; Saad et al 2025; Shahimi et al 2021). Despite regulatory restrictions on certain antibiotics, residues have been detected in Vietnamese aquaculture products (MARD 2022), which may facilitate the persistence and horizontal transfer of high-risk resistance genes such as *mcr* and *cfr*, posing threats to both veterinary and human medicine (Zhu et al 2020). Environmental salinity may also indirectly influence antibiotic efficacy by promoting biofilm formation and cell aggregation, which can limit antibiotic diffusion and enhance bacterial survival (Hu et al 2022). These adaptive responses are particularly relevant in brackish aquaculture systems in Central Vietnam, where seasonal salinity fluctuations may favor the persistence of antibiotic-tolerant *Vibrio* populations. From a management perspective, these findings underscore the importance of salinity-aware disease control strategies, prudent antibiotic use, and routine resistance monitoring to mitigate AMR risks in aquaculture. At the interface between aquatic environments and the food chain, such measures are also essential for reducing the potential dissemination of resistance determinants affecting public health (WHO 2015; Rescan et al 2025). In this study, phenotypic resistance was assessed in all isolates, whereas genotypic screening was conducted on a single representative isolate due to the high similarity in phenotypic profiles and resource constraints. While this approach is commonly adopted in studies involving isolates from a single host population or outbreak, several limitations should be acknowledged. The number of isolates analyzed was limited, resistance gene expression was not quantified, and transcriptomic or genomic analyses were not performed. Future studies incorporating larger isolate collections and transcriptomic or whole-genome approaches will be necessary to more precisely elucidate salinity-driven antibiotic tolerance and strain-specific variability in marine *Vibrio* populations.

Conclusions. This study highlights AMR in *V. alginolyticus* isolated from diseased *S. ocellatus* cultured in Central Vietnam. Although phenotypic resistance was limited, the isolates harbored a broad range of resistance genes associated with antibiotics commonly used in aquaculture as well as in veterinary and human medicine, indicating a considerable potential for resistance dissemination. Variations in environmental salinity were associated with changes in bacterial antibiotic tolerance, suggesting that physicochemical conditions in aquaculture systems may influence ARM expression. These findings emphasize the need for routine resistance monitoring, prudent antibiotic use, and improved management practices in aquaculture. Future studies incorporating transcriptomic or genomic approaches and long-term surveillance will be essential to better understand and mitigate ARM in aquatic production systems.

Ethical approval. All experimental procedures involving animals were conducted in accordance with the guidelines of the Hue University Animal Ethics Committee and were approved under certificate number HUVN0054 (December 10, 2024). All efforts were made to minimize animal suffering.

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Conflict of interest. The authors declare that there is no conflict of interest.

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