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Status of the antimicrobial resistance in *Vibrio* from *Penaeus monodon* shrimp culture system of Kerala, India

S. Paila[1,2], D. Pillai[2], E.N. Sumathi[1], V. Sivam[1], J.C. George[1], T.C. Joseph[1], M.M. Prasad[3], M.R. Badireddy[3] and M. Vaiyapuri[1]*

[1]*Microbiology Fermentation and Biotechnology Division, ICAR-Central Institute of Fisheries Technology, Cochin-682 029, India*
[2]*Department of Aquatic Animal Health Management, Kerala University of Fisheries and Ocean Studies, Cochin-682 506, India*
[3]*Visakhapatnam Research Centre of CIFT, Visakhapatnam-530 003, India*

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***Corresponding Author Email:** murugadascift81@gmail.com ***ORCiD:** https://orcid.org/0000-0001-5807-9290

Abstract

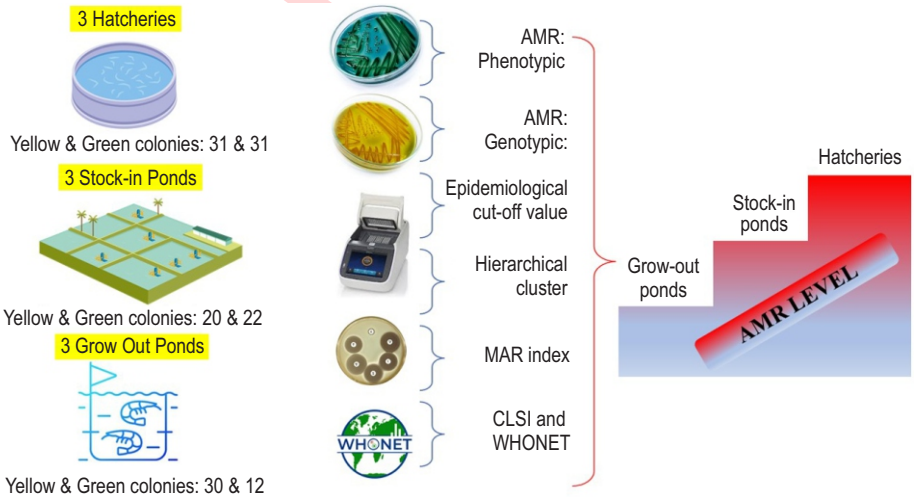
Aim: To estimate the antimicrobial resistance (AMR) in *Vibrio* associated with *Penaeus monodon* from shrimp hatcheries and aquaculture farms located in Kerala, India, and further estimate the AMR between Sucrose Fermenting Vibrio (SFV) and Sucrose Non-Fermenting Vibrio (SNFV).

Methodology: Samples (n = 24) from grow-out, stocking ponds and hatcheries were collected and enumerated for total *Vibrio*, SNFV and SFV. Molecularly confirmed *Vibrio* sp., (n=115) were taken for antibiotic susceptibility test (AST) by disc diffusion assay and AMR was interpreted. Multiple Drug Resistance (MDR), Multiple Antibiotic Resistance (MAR) index, Epidemiological cut-offs were also estimated.

Results: The study demonstrated that 64% *Vibrio* from shrimp culture system showed resistance towards ampicillin, followed by cefoxitin (60%), ciprofloxacin (40%), cefotaxime (20%) and others below 15%. MDR was observed at 10.4%. AMR pattern found among *Vibrio* 15 types. MAR index above 0.2 was found in stocking pond. Antibiotic resistant determinants bla_{tem} and *tet B* for β-lactam and tetracycline resistance were only detected in the study.

Interpretation: *Vibrio* from aquaculture farms are more susceptible than hatcheries, and also *Vibrio* present in the environment are more resistant than those found in animals.

Key words: Antimicrobial resistance, Shrimp aquaculture systems, Sucrose non-fermenting *Vibrio*



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Introduction

Aquaculture is one of the fastest-growing food sectors in the world, contributing significantly to food and nutritional security. The contribution of aquaculture to global aquatic animal production has risen steadily, and for the first time, aquaculture surpassed capture fisheries in 2022 (FAO, 2024). Asian countries have emerged as major aquaculture producers, and shrimp aquaculture in India has steadily transformed into a profitable industry. To meet the increasing demand, many countries have adopted intensive shrimp farming practices to enhance production. However, intensive aquaculture inevitably increases disease incidence, leading to economic losses, primarily due to bacterial etiologies, which requires the use of antimicrobial agents for treatment (Ninawe *et al.,* 2017). This practice potentiates the emergence of antimicrobial resistance (AMR) in bacteria associated with farmed aquatic animals and the aquatic environment (Akinbowle *et al.,* 2006).

Vibriosis, caused by *Vibrio* spp., is responsible for significant mortality and severe economic losses in the shrimp industry, affecting stages from broodstock and hatcheries to grow-out ponds (Peeralil *et al.,* 2020; Vaiyapuri *et al.,* 2021a; Nilavan *et al.,* 2025). While most *Vibrio* species are regarded as non-pathogenic commensals, a few are pathogenic. Antibiotics are commonly used for prophylactic or therapeutic purposes in hatcheries, particularly when larval development is impaired (Srinivasan and Ramaswamy, 2009). The emergence and dissemination of AMR are currently among the major public health concerns worldwide, resulting due to lack of new antibiotic discoveries and the declining efficacy of existing antimicrobials in treating serious infections (Nekouei *et al.,* 2018).

AMR bacteria can be transferred directly or indirectly from animals to humans through the consumption of contaminated seafood or water (Visnuvinayagam *et al.,* 2015; 2017). It is estimated that by the year 2050, AMR could lead to 10 million deaths annually, resulting in a global economic loss of 100 trillion dollars. Considering the significance of *Vibrio* spp. and antimicrobial resistance in shrimp aquaculture sector. The present study aimed to assess the incidence of AMR in *Vibrio* spp. across different components of shrimp aquaculture systems *viz.,* hatcheries, stocking ponds, and grow-out ponds—randomly selected from various locations in Thrissur district, Kerala.

Materials and Methods

Collection of samples: Sediment, water and shrimp samples (n = 24) were collected from different shrimp aquaculture systems, *viz.,* hatcheries, stocking ponds, and grow-out ponds in and around Thrissur district, Kerala, India. Three shrimp and three water samples were collected from each of the three hatcheries. From the stocking and grow-out ponds, three samples each of shrimp, sediment and water were collected. Sediment and water samples were aseptically collected in sterile sample containers, while shrimp samples were collected in sterile zip-lock bags. All samples were transported to the laboratory under chilled conditions and processed immediately for further analysis.

Enumeration and isolation of *Vibrio* species: One gram of each sediment or shrimp sample, or 1 ml of each water sample, was aseptically transferred into separate tubes containing 9 ml of sterile phosphate-buffered saline (PBS). The samples were thereafter, subjected to tenfold serial dilutions. From each dilution, 0.1 ml was spread-plated onto the respective agar media. The total heterotrophic count (TPC) was determined using Marine Agar (2216, Difco, USA), and the total *Vibrio* count (TVC) was determined using thiosulfate–citrate–bile salts–sucrose (TCBS) agar (Reasoner, 2004). All plates were incubated at 30 ± 1 °C for 18 hr. After incubation, TPC and TVC were enumerated. Total Sucrose-fermenting *Vibrio* (TSFV) were calculated based on the number of yellow colonies, while Total Sucrose non-fermenting *Vibrio* (TSNFV) were calculated based on the number of green colonies on TCBS agar plates used for TVC (Farmer *et al.,* 2005).

Identification, purification and maintenance of isolated *Vibrio*: Based on the morphological characteristics, 3–5 SFV and SNFV colonies were randomly selected from TCBS plates, and the isolates were purified on TCBS agar. The purified isolates were preserved on Brain Heart Infusion (BHI) slants supplemented with 1% NaCl or in 20% glycerol at –80 °C. For presumptive genus-level identification, the bacterial isolates were subjected to Gram staining, catalase and oxidase tests, and amino acid hydrolysis assays (Pei *et al.,* 2017).

Molecular identification of *Vibrio* species: Crude DNA was extracted from 1 ml of overnight-grown culture placed in sterile 1.5 ml microfuge tubes. The cell pellet was harvested by centrifugation at 8,000 rpm for 10 min and washed twice with 500 µl of 1× TE buffer. The tubes were then incubated on a dry bath at 95°C for 10 min and immediately transferred to cold storage. The supernatant was transferred to a separate tube and stored as template DNA for future use. PCR amplification was carried out in a thermocycler (Applied Biosystems) for 35 cycles (Tarr *et al.,* 2007). The amplified PCR products (10 µl) were analyzed on a 2% agarose gel using a submarine electrophoresis system (1× TAE). The gel was subsequently visualized using a gel documentation system (Syngene, UK).

Estimation of antimicrobial resistance: *Vibrio* cultures grown overnight in BHI broth at 30 °C were adjusted to a turbidity of 0.5 McFarland standard and swabbed onto Mueller–Hinton Agar supplemented with 1% NaCl, following the Kirby–Bauer disc diffusion method (CLSI, 2016). Twelve antibiotics representing eight classes were tested, and the plates were incubated at 30 °C for 16–20 hr. The inhibition zones for each *Vibrio* isolate were interpreted according to CLSI Guidelines (2016). The antibiotics used in this study were: ampicillin 10 µg, amoxicillin–clavulanic acid 30 µg, cefepime 30 µg, cefotaxime 30 µg, cefoxitin 30 µg, ceftazidime 30 µg, meropenem 10 µg, gentamicin 10 µg, tetracycline 30 µg, ciprofloxacin 5 µg, trimethoprim–sulfamethoxazole 25 µg, and chloramphenicol 30

µg (Vaiyapuri *et al.,* 2021b). Multiple Drug Resistance (MDR), and Multiple Antibiotic Resistance (MAR) index was estimated (Magiorakos *et al.,* 2012). The Epidemiological Cut off value (Ecoff) was established using the normalised resistance interpretation approach for classifying wild type (WT) or non-wild type (NWT) (Kronvall *et al.,* 2011).

PCR for detection of ARGs: *Vibrio* sp. that showed phenotypic resistance to cefpodoxime, ceftazidime, aztreonam and cefotaxime were tested for bla_{tem}, bla_{shv}, bla_{ctxm1}, bla_{ctxm2}, bla_{ctxm9}, $bla_{ctxm8/25}$, bla_{oxa}, genes, specific for ESBL detection (Dallenne *et al.,* 2010). Isolates showing phenotypic resistance towards tetracycline, cotrimoxazole, chloramphenicol, ciprofloxacin were tested for (*tetA* and *tetB*), (*sul1, sul2* and *dfrA1, dfrA18*), (*catA1* and *catA2, catA3* and *catB3*), and *qnr* genes, respectively (Hochhut *et al.,* 2001; Chuanchuen and Padungtod, 2009; Momtaz *et al.,* 2012; Kim *et al.,* 2013).

Statistical analyses: The hierarchical cluster technique was used to analyse the AMR pattern indicating the absence or presence of resistance. A binary squared Euclidean distance matrix was created from AMR data between two cultures. SPSS version 16 was used to build a dendrogram based on a similarity matrix. The mean values of TPC, TVC, TSFV and TSNFV were compared using SAS system version 9.3 in generalized least square methods between animals and water, between stocking and grow out farms and hatcheries.

Results and Discussion

Vibriosis is one of the most common diseases affecting shrimp. The frequent and inappropriate use of antibiotics, often without professional supervision, to control infections can promote the selection of antimicrobial-resistant (AMR) bacteria and facilitate the spread of antibiotic resistance genes (ARGs) within the bacterial population in the environment. *Vibrio* spp. are ubiquitous in aquatic ecosystems and form a part of the normal flora of coastal seawaters, seafood including shrimp, and aquaculture systems (Rao and Surendran, 2013). The present study aimed to assess the incidence of AMR in *Vibrio* spp. across different components of shrimp aquaculture systems *viz.,* hatcheries, stocking ponds, and grow-out ponds—randomly selected from various locations in Thrissur district, Kerala.

The TPC, TVC, TSFV and TSNFV counts from water, sediment and shrimp samples, as well as the numbers of SNFV and SFV randomly selected for the study, are presented in Table 1. Animals from hatcheries showed TPC values of 4.7–5 log CFU g^{-1}, while stocking and grow-out farms recorded 5.04–6.47 log CFU g^{-1} and 3.65–5.30 log CFU g^{-1}, respectively. All 115 presumptive *Vibrio* isolates from TCBS were confirmed biochemically and molecularly by PCR, producing an amplicon of 663 bp. Approximately, 70% of the isolates were Sucrose-fermenting *Vibrio,* while the remainings were Sucrose non-fermenting. TPC values in animal samples did not exceed >7 log CFU g, indicating that the overall quality of the animals and the pond environment was satisfactory. In general, the TVC of stocking ponds was higher than that of grow-out ponds and hatcheries. Sucrose non-fermenting *Vibrio* were not detected in hatchery samples whereas Sucrose-fermenting *Vibrio* counts ranged from 0 to 3 log CFU g^{-1} in animal samples and 0 to 4.3 log CFU m l^{-1} in water. Similarly, pathogenic SNFV species, including *Vibrio parahaemolyticus, V. mimicus* and *V. vulnificus,* were not detected in hatchery samples (Smith and König, 2010). Low TVC counts (<3 log CFU m l^{-1}) have been reported in shrimp ponds (Heenatigala *et al.,* 2023) whereas higher TVC levels in hatchery water (3 log CFU m l^{-1}) compared to pond water (2 log CFU m l^{-1}) have also been documented (Rao and Surendran, 2013).

The mean counts of TPC (4.30–5.04 log CFU g^{-1}), TVC (1.61–2.93 log CFU g^{-1}), TSFV (1.53–2.38 log CFU g^{-1}), and TSNFV (0.32–1.33 log CFU g^{-1}) between animal and water samples did not vary significantly ($P \leq 0.05$) among the three components of the shrimp aquaculture system, namely hatchery, stocking, and grow-out ponds. However, a significant difference($P \leq 0.05$) in TPC values between animal and water samples was observed in the stocking and grow-out ponds, with counts ranging from 3.02 to 5.87 log CFU g^{-1}. Similarly, a significant difference ($P \leq 0.05$) in TSNFV values was observed between the grow-out pond and hatchery samples, ranging from 0.30 to 1.64 log CFU g^{-1}. The water quality of hatcheries, stocking, and grow-out ponds was within the following ranges: dissolved oxygen 12–14, 5.8–7.5, and 5–9.5 mg l^{-1}, respectively; salinity 18–20, 5.6–6.8, and 3–12 ppt, respectively; and pH 7.8–8.1, 7.0–7.1, and 7.4–8.0, respectively. Except for the pH of stocking ponds and the salinity range of grow-out ponds, all water quality parameters were within optimal limits for shrimp aquaculture. Minor variations in water quality, as well as differences in season, geographical location, and transportation of post-larvae, may contribute to the observed variations in microbial counts (Heenatigala *et al.,* 2023).

The results of the AMR analysis among *Vibrio* isolates are shown in Fig. 1 (a–f). The study revealed that 64% of the *Vibrio* isolates from all types of shrimp culture systems were resistant to ampicillin. The resistance pattern followed the order: 60% for cefoxitin, 40% for ciprofloxacin, 20% for cefotaxime, 15% for amoxicillin and co-trimoxazole, and less than 5% for ceftazidime and cefepime. The detailed AMR patterns and variations are presented in Table 2. Overall, resistance was observed against one to six antibiotics among the isolates. In the present study, variations in AMR patterns among *Vibrio* species may be attributed to the presence of distinct strains. *Vibrio* isolates were mostly resistant to ampicillin, followed by cefoxitin. Although ampicillin is not routinely used in shrimp culture, the majority of Vibrio exhibited resistance, likely due to intrinsic resistance mechanisms (Akinbowale *et al.,* 2006).

Even though farm managers reported no antibiotic usage during the current survey, the presence of AMR *Vibrio* in shrimp could have originated from post-larvae, as hatchery and stocking pond samples generally showed slightly higher resistance than

Table. 1: Details of microbiological count in samples from shrimp aquaculture system and colonies selected for antimicrobial resistance

Hatchery/ Farm	Sample type	TPC (log CFU g^{-1} or m l^{-1})	TVC (log CFU g^{-1} or m l^{-1})	TSFV (log CFU g^{-1} or m l^{-1})	TSNFV (log CFU g^{-1} or m l^{-1})	No. of colonies taken	Colony's identity	Total colonies selected
H1	Animal	5	Nil	Nil	Nil	0	-	Hatchery - 31
	Water	5.3	3	3	Nil	10 SFV	H1WY1 to H1WY10	colonies
H2	Animal	4.47	3	3	Nil	8 SFV	H2AY1 to H2AY8	Hatchery animals
	Water	5.95	4.34	4.34	Nil	10 SFV	H2WY1 to H2WY10	SFV – 11
H3	Animal	4.77	2.47	2.47	Nil	3 SFV	H3AY1 to H3AY3	Hatchery Water
	Water	5.30	Nil	Nil	Nil	0	0	SFV – 20
S2	Animal	5.04	3.30	3.17	2.69	5 SFV / 5 SNFV	S2AY1 to S2AY5/ S2AG1 to S2AG5	Stocking ponds –42 colonies
	Water	5.65	2.30	2.30	Nil	2 SFV	S2WY1, S2WY2	Stocking pond
	Sediment	5.04	2.69	2.69	Nil	5 SFV	S2SY1- S2SY5	animals SFV - 20
S3	Animal	6.17	3.30	2.90	2.77	5 SFV / 5 SNFV	S3AY1 to S3AY5/ S3AG1 to S3AG5	SNFV – 10 Stocking pond
	Water	5.41	3	2.47	2.30	3 SFV / 2 SNFV	S3WY1 to S3WY3/ S3WG1 to S3WG2	water SFV - 5 SNFV – 2
	Sediment	4.90	Nil	Nil	Nil	0	0	Stocking pond
S4	Animal	6.47	5.46	4.44	Nil	10 SFV	S4AY1 to S4AY10	sediment SFV - 5
	Water	6.48	Nil	Nil	Nil	0	0	
	Sediment	ND	ND	ND	ND	ND	ND	
G1	Animal	5.30	2.84	Nil	2.84	7 SNFV	G1AG1-G1AG7	Grow out ponds
	Water	5.30	3.07	2.84	2.69	5 SFV, 5 SNFV	G1WY1-G1WY5, 1WG1-G1WG5	–42 colonies Grow out pond
	Sediment	4.90	3	2.77	2.60	6 SFV, 4 SNFV	G1SY1-G1SY6, G1SG1-G1SG4	animals SFV - 9 SNFV–13 Grow
G3	Animal	4.47	3.64	3.60	2.60	6 SFV, 4 SNFV	G3AY1-G3AY6, G3AG1-G3AG4	out pond water SFV-5 SNFV–5
	Water	Nil	Nil	Nil	Nil	0	_	Grow out pond
	Sediment	4	Nil	Nil	Nil	0	_	sediment SFV –
G4	Animal	3.65	2.69	2.47	2.30	3 SFV, 2 SNFV	G4AY1-G4AY3, G4AG1, G4AG2	6 SNFV – 4
	Water	Nil	Nil	Nil	Nil	0	_	
	Sediment	3.60	Nil	Nil	Nil	0	_	

Note: TPC- Total Heterotrophic Plate Count; TVC-Total Vibrio Count; TSFV-Total Sucrose Fermenting Vibrio Count; TSNFV – Total Sucrose Non-Fermenting Vibrio count; ND- Not determined; Nil- No colonies were observed. H – Hatchery, S – Stocking ponds, G-Grow-out ponds

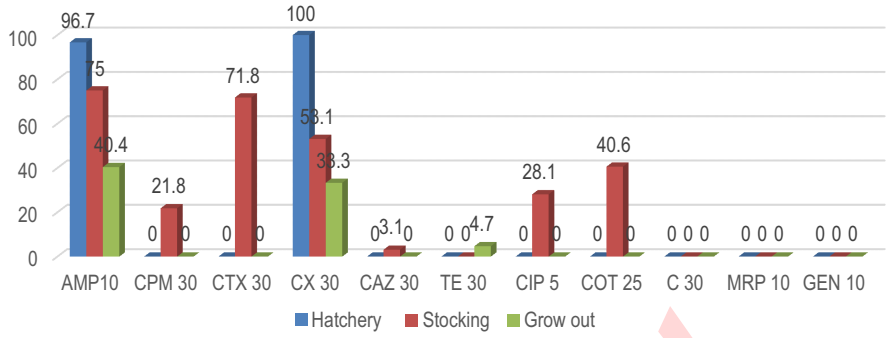
grow-out pond samples. Resistant *Vibrio* were particularly observed in animals from hatcheries and stocking ponds. The use of antibiotics prophylactically during larval and post-larval rearing, including medicated feed or to maintain water quality, may contribute to the development of AMR. However, the overall levels of AMR in the shrimp aquaculture system were relatively low. In particular, resistance to tetracycline was negligible. These findings are consistent with previous studies (Sundaramanickam *et al.,* 2015). While true prophylactic use of antibiotics in aquaculture in the absence of disease is uncommon, it occurs more frequently in hatcheries (Zhang *et al.,* 2011).

For example, in Bangladesh, a single shrimp hatchery used 80 kg of antibiotics per production cycle (Hinchcliffe *et al.,* 2018), highlighting that post-larval stages up to stocking represent a critical point for implementing AMR control strategies. Distinct variations in AMR have been observed in *Vibrio* isolates from farms, retail markets and larval tanks, as well as between different *Vibrio* species (*V. parahaemolyticus* versus other *Vibrio*

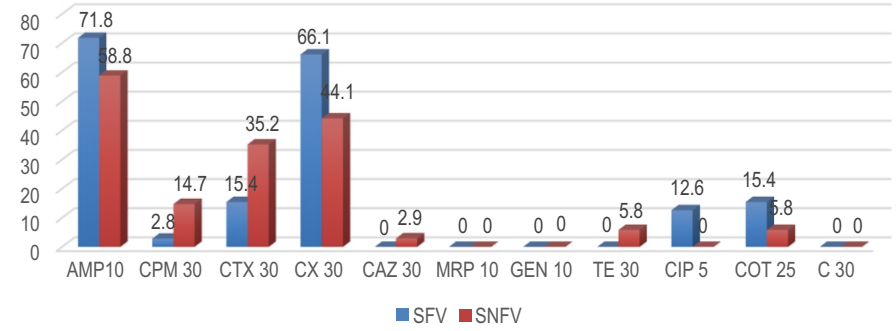
Table. 2: AMR patterns observed between hatchery, stocking and grow out ponds

Grow out pond	Hatchery	Stocking
CX	CX	Stocking
TE	AMP-CX	AMP
AMP		CTX-CX
AMP-CX		AMP-CIP
		AMP-CPM-CTX
		AMP-CTX-CAZ
		AMP-CTX-CIP
		AMP-CPM-CX
		AMP-CTX-CX
		AMP-CTX--CIP-COT
		AMP-CPM-CTX-CX
		AMP-CTX-CX-COT
		AMP-CTX-CX-CIP-COT
		AMP-CPM-CTX-CX-CIP-COT
No MDR strain 42 *Vibrio*	No MDR strains 32 *Vibrio*	12 MDR strain 33 *Vibrio*

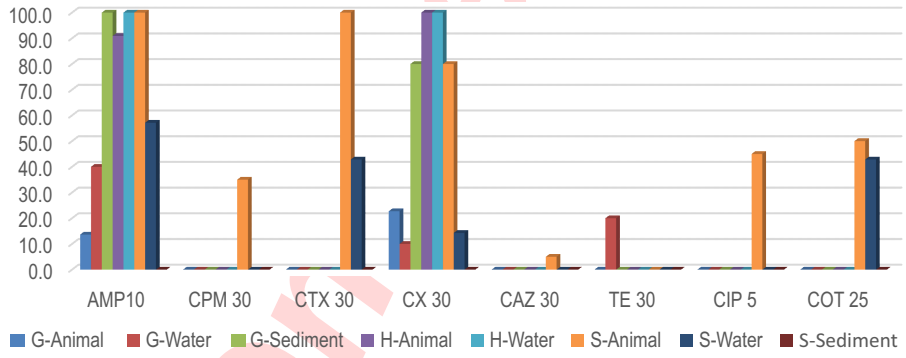
(a)


96.7
75
40.4
21.8
71.8
100
58.1
38.3
3.1
4.7
28.1
40.6
AMP10
CPM 30
CTX 30
CX 30
CAZ 30
TE 30
CIP 5
COT 25
C 30
MRP 10
GEN 10
Hatchery
Stocking
Grow out

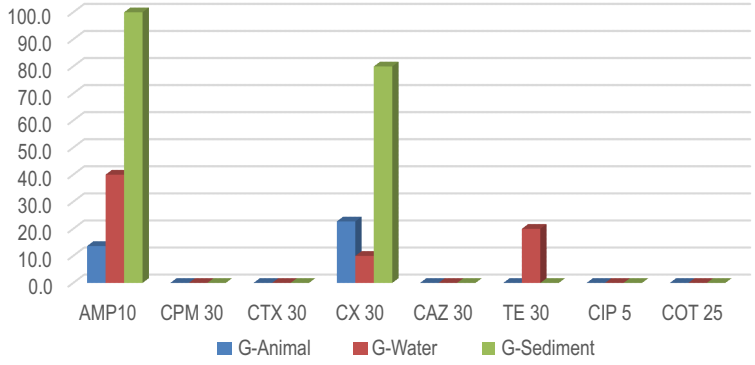
(b)


71.8
58.8
2.8
14.7
15.4
35.2
66.1
44.1
2.9
5.8
12.6
15.4
5.8
AMP10
CPM 30
CTX 30
CX 30
CAZ 30
MRP 10
GEN 10
TE 30
CIP 5
COT 25
C 30
SFV
SNFV

(c)


AMP10
CPM 30
CTX 30
CX 30
CAZ 30
TE 30
CIP 5
COT 25
G-Animal
G-Water
G-Sediment
H-Animal
H-Water
S-Animal
S-Water
S-Sediment

(d)


AMP10
CPM 30
CTX 30
CX 30
CAZ 30
TE 30
CIP 5
COT 25
G-Animal
G-Water
G-Sediment

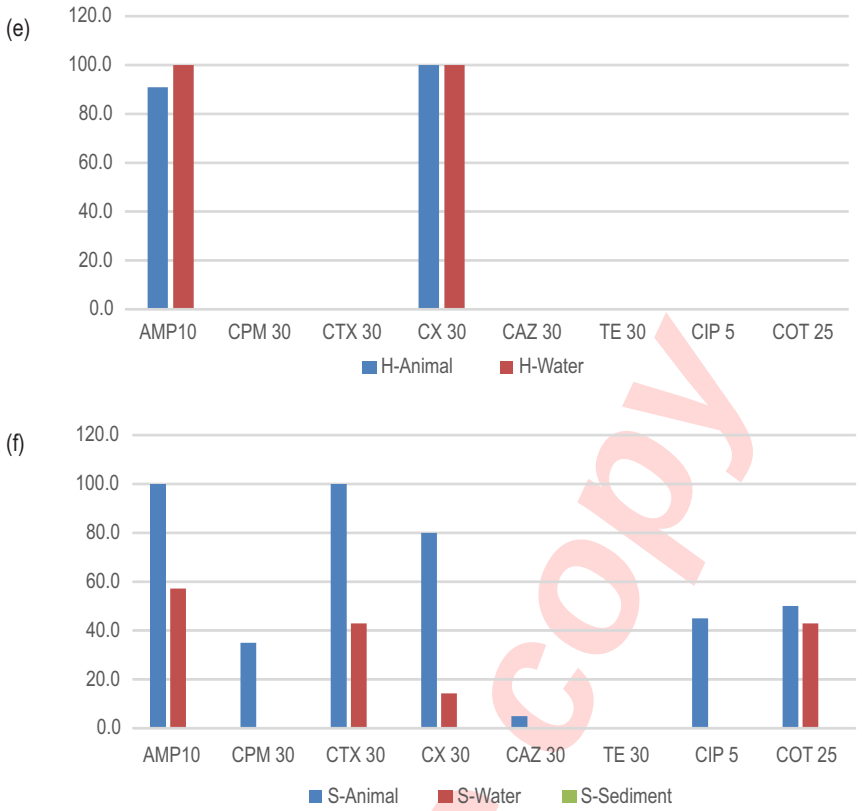


Fig. 1: Status of antimicrobial resistance (AMR) in *Vibrio* from *P. monodon* culture system; (a) AMR observed in *Vibrio* between hatchery, stocking and grow out ponds; (b) AMR variation observed between SNFV and SFV; (c) AMR observed between animal, water, sediment from hatchery, stocking and grow out Vibrio; (d to f) depicts the AMR observed between grow out, hatchery and stocking pond respectively.

species) and across countries, including Ecuador, Malaysia and Bangladesh, for antibiotics such as ampicillin, tetracycline, aminoglycosides, ciprofloxacin, and sulfonamides (Otta *et al.,* 2001; Letchumanan *et al.,* 2015; Sperling *et al.,* 2015; Heenatigala *et al.,* 2023). Although tetracycline has been recommended for aquaculture use and some studies have reported higher resistance in *Vibrio*, in this study minimal resistance, was observed, indicating its rare use in *Penaeus monodon* culture practices (Pan *et al.,* 2013). Among the *Vibrio* isolates from the shrimp aquaculture system, 10.4% were found to be multidrug-resistant. The majority of MDR strains were detected in stocking pond samples. The calculated multiple antibiotic resistance index showed that, overall, the MAR index was below the 0.2 threshold. However, isolates from stocking ponds exceeded threshold whereas those from grow-out ponds did not. Effective control strategies in *Penaeus monodon* aquaculture should, therefore, focus on the pre-stocking period rather than the culture ponds. Previous studies have reported higher levels of MDR resistance (26–90%) and MAR indices, exceeding 0.2 in 100% of Vibrio isolates from shrimp aquaculture systems (Kitiyodom *et al.,* 2010; Sudan *et al.,* 2023; Prabina *et al.,* 2023). Determination of ECOFF values for the *Vibrio* isolates revealed an increase in the number of wild-type strains in the aquaculture farms. The ECOFF values for AMC, AMP, CAZ, CHL, CIP, CPD, CTX, CX, GEN, MRP, SXT, and TE were ≥22 mm, ≥10 mm, ≥21 mm, ≥22 mm, ≥26 mm, ≥19 mm, ≥21 mm, ≥11 mm, ≥16 mm, ≥24 mm, ≥19 mm, and ≥17 mm, respectively, indicating a shift in the size of inhibition zone.

These results suggest that the majority of the strains belong to the wild-type population when ECOFF values are applied, compared to the clinical breakpoints recommended by CLSI or EUCAST. Therefore, extensive data collection is required to establish ECOFF Guidelines for *Vibrio* associated with the shrimp aquaculture environment (Kronvall *et al.,* 2011). The study revealed that among *Vibrio* isolates from the stocking pond (n = 31) showing phenotypic resistance to third-generation cephalosporins and monobactams—specifically cefpodoxime, ceftazidime and cefotaxime - only one SFV isolate (S2WY1) carried the bla_{tem} gene. This suggests that the isolate may be resistant as a broad-spectrum β-lactamase or an extended-spectrum β-lactamase-producing *Vibrio*. Two SFV isolates from the grow-out pond (G1WY1 and G1WY2) exhibiting phenotypic tetracycline resistance were found to carry the *tetB* gene, while other tetracycline-resistant isolates may possess resistance mechanisms other than tet*A* or tet*B*. None of the isolates tested

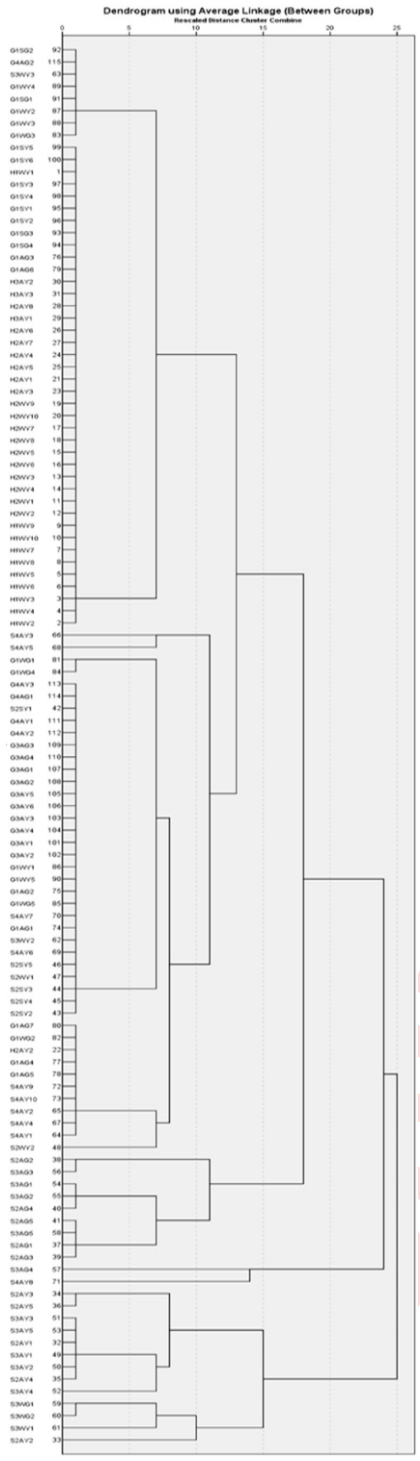


Fig. 2: Association analysis of AMR with aquaculture system.

positive for other antibiotic resistance genes (ARGs), including *viz., bla*$_{ctxm1}$, *bla*$_{ctxm2}$, *blactxm9, bla*$_{ctx8/25}$, *bla*$_{shv}$, *bla*$_{oxa}$, *sul1, sul2, dfrA1, dfrA18, catA1, catA2, catA3 and catB3 and qnr.* Although Class I integron genes have been detected in sulfonamide-resistant *Vibrio* from marine shrimp (Kitiyodom *et al.,* 2010), they were not detected in the present study. This indicates that sulfonamide resistance mediated by folate synthesis inhibitors may occur through alternative mechanisms. Research on tetracycline resistance genes in aquaculture farms near the Baltic Sea detected *tetA*, *tetC*, *tetH*, *tetM*, *tetE*, *tetG*, and *tetW* in ponds despite the absence of tetracycline use for over six years (Tamminen *et al.,* 2011). Cluster analysis based on AMR patterns revealed that the isolates grouped into five main clusters, labelled A to E (Fig. 2). Isolates from hatcheries and grow-out pond 1 clustered together, while stocking pond and other grow-out pond isolates formed distinct clusters, indicating that resistance patterns in stocking ponds differ from those in hatcheries and grow-out ponds. This is the first study to include Vibrio isolates from all stages of the *Penaeus monodon* shrimp culture system for AMR determination.

Overall, AMR and ARGs were detected at very low levels. However, this concern is heightened by the fact that many pathogens present in aquaculture ponds and hatcheries, such as *Vibrio* and *Aeromonas* species, are endemic to surrounding marine and estuarine environments. Consequently, resistant bacteria released into these environments have the potential to become dominant strains. This risk is further amplified by high levels of antibiotic pollution in areas surrounding aquaculture facilities, with increasing evidence that farms themselves contribute to this pollution through the discharge of water and pond sediments (Akinbowale *et al.,* 2006). There is considerable variation in the data on AMR and the identification of ARGs within shrimp aquaculture systems. This study highlights the urgent need to explore alternatives to antibiotics that have been evaluated for ecotoxicity, as part of improved aquatic animal health management strategies for controlling *Vibrio* in aquaculture. Given the high frequency of infections on local shrimp farms and the concerning rise in AMR, antimicrobial use (AMU) requires comprehensive research across all components of shrimp aquaculture systems.

The use of appropriate therapeutic antibiotic dosages, along with alternative management strategies, can help mitigate potential environmental and human health impacts (Nogueira-Lima *et al.,* 2006). There is growing interest in bacteriophage-based biocontrol of *Vibrio* in shrimp aquaculture (Benala *et al.,* 2023), as well as other approaches, including bacteriocins, phytomolecules, and natural antimicrobials (Vaiyapuri *et al.,* 2021a). AMR mitigation in the shrimp aquaculture sector should follow a One Health approach, as aquatic ecosystems receive terrestrial inputs, including human and animal waste, and the environment is an integral component of aquaculture. Environment acts as a sink for the development and spread of AMR through ARGs (Mothadaka *et al.,* 2023; Vaiyapuri *et al.,* 2023; Sanjeev *et al.,* 2023).

Future studies should focus on identifying the mechanisms of resistance in phenotypically resistant *Vibrio*, standardizing procedures for AMR determination, and incorporating antibiotic residue analyses. Such approaches will improve the interpretation of antimicrobial resistance in *Vibrio*

within shrimp aquaculture systems and provide better insight into the relationship between AMR and antimicrobial usage.

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