Studies on the Prevalence and Antibiotic Resistance Patterns of Bacteria (Vibrio) Isolated from Fish and Shrimp

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Abstract- Antibiotic resistance in aquaculture is an emerging concern that threatens both animal health and public safety.This study investigated antibiotic resistance trends of Vibrio species isolated from aquaculture environments in the Nellore District, Andhra Pradesh. Eight distinct Vibrio species were identified from fish and shrimp samples collected from various ponds and water channels: Vibrio alginolyticus, Vibrio mimicus, Vibrio fluvialis, Vibrio harveyi, Vibrio splendidus, Vibrio vulnificus, Vibrio cholerae, and Vibrio parahaemolyticus. Morphological, biochemical, and antibiotic sensitivity tests were conducted to confirm their identities and resistance profiles. The results indicated resistance to several commonly used antibiotics, including oxytetracycline, erythromycin, and ciprofloxacin, with some species exhibiting complete resistance to specific antibiotics. While chloramphenicol and furazolidone demonstrated broad effectiveness, their use is restricted due to potential health risks. Gentamycin showed moderate effectiveness, and ampicillin exhibited limited efficacy. These findings highlight the prevalence of antibiotic-resistant Vibrio species in aquaculture, underscoring the necessity for responsible antibiotic use and the exploration of alternative disease control methods. This study provides crucial data for informing future research and policy aimed at combating antimicrobial resistance in aquaculture.

Indexed Terms- Antibiotic resistance, Vibrio species, Aquaculture, Nellore District, Andhra Pradesh, Sensitivity testing, Alternative disease control, Public health.

I. INTRODUCTION

An essential part of the world's food supply is produced via aquaculture, providing a significant portion of the animal protein consumed by humans. The rapid growth of aquaculture, particularly in coastal countries like India, China, and Vietnam, has contributed greatly to economic development, domestic consumption, and international exports. In India, aquaculture supports millions of livelihoods. However, the industry faces numerous challenges, the most critical being the frequent outbreaks of infectious diseases caused by bacterial pathogens. Among these, Vibrio species pose a substantial threat due to their prevalence, pathogenicity, and increasing resistance to antimicrobial agents (Kumar et al., 2016).

Vibrio species are ubiquitous in marine, estuarine, and freshwater environments, where they play essential roles in nutrient cycling. Despite being part of the natural microbiota, several Vibrio species are opportunistic pathogens which may infect many different kinds of fish and prawns, among many other animals in water. These bacteria cause severe diseases in aquaculture, leading to high mortality rates and significant economic losses. Additionally, some Vibrio species are zoonotic, capable of infecting humans through the consumption of contaminated seafood, causing gastrointestinal illnesses and, in severe cases, systemic infections (Silvester et al., 2019). The most notable species involved in both aquaculture and human infections are *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio alginolyticus*. These pathogens can cause vibriosis in fish and shrimp, manifesting as hemorrhaging, skin lesions, and internal organ damage. In intensive farming systems, where fish and shrimp are densely stocked, vibriosis outbreaks can spread rapidly, decimating entire stocks (Jeyasanta et al., 2017).

Antibiotics are widely used in aquaculture, both therapeutically and prophylactically, to control bacterial infections. In many cases, antibiotics have also been used at sub-therapeutic levels for growth promotion, a practice now banned in many regions. Vibrio species, in particular, have demonstrated the capacity to develop resistance to multiple antibiotics, including commonly used drugs like oxytetracycline, erythromycin, and ciprofloxacin (Elmahdi et al., 2016). The rise of antibiotic resistance in Vibrio species presents a dual threat. First, it diminishes the effectiveness of antibiotics in treating infections in aquaculture, leading to higher mortality rates and financial losses for farmers. Second, these resistant bacteria can transfer their resistance genes to other bacterial populations, including human pathogens, through horizontal gene transfer mechanisms such as plasmids, transposons, and integrons. This significantly increases the risk of untreatable infections in humans, exacerbating the global health crisis of antimicrobial resistance (AMR) (Srinivasan et al., 2009). Given the growing concern over antibiotic resistance, there is a pressing need for comprehensive studies that assess the prevalence of antibiotic-resistant Vibrio species in aquaculture environments. These studies provide crucial data on the distribution of resistant bacteria and their resistance patterns, which can inform disease management strategies and guide regulatory policies on antibiotic use in aquaculture (Shakerian et al., 2018).

II. MATERIALS AND METHODS

2.1 Geographic Area of Study and Sample Collection

This study collected 100 fish and shrimp samples from aquaculture ponds and water channels in key villages of Nellore District, including Gangapatnam, Somarajupalli, and Nidimusali in Indukurpet, as well as Venkannapalem, Amuluru, and Eduru in TP Guduru. Additional samples of *Oreochromis mossambicus* were gathered from Tippaguntapalem, Pudipatri, and Kolanakudur in Kandaleru Creek.

2.2 Isolation and Enumeration of Vibrio Species

Gut samples were homogenized in saline and subjected to serial dilution (10^{-1} to 10^{-5}). Aliquots (0.1 ml) were inoculated onto Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar, incubated at 32°C for 24-48 hours. Colonies were counted and categorized by color: yellow colonies indicated *Vibrio parahaemolyticus* and green colonies indicated *Vibrio alginolyticus*. Selected colonies were purified on Tryptic Soy Agar (TSA) for further analysis (Raja et al.,2017).

2.3 Biochemical Characterization of Vibrio Isolates

The biochemical characterization of Vibrio isolates involved several tests to confirm their identity. Initially, Gram staining was performed to verify that all isolates were Gram-negative. Colony morphology on TCBS agar was documented, focusing on color and size. The motility of the isolates was assessed using the hanging drop method, while the oxidase test confirmed the presence of Cytochrome C oxidase. Additional tests included indole production from tryptophan, citrate utilization as a carbon source, and identification of acetoin through the Voges-Proskauer test. The methyl red test assessed acid production from glucose fermentation, and the lysine decarboxylase test evaluated lysine metabolism. The ONPG test identified β-galactosidase activity, and halophilism was tested by inoculating isolates in varying salt concentrations. Finally, acid production from D-cellobiose was monitored to complete the biochemical profile of the Vibrio isolates (Eid et al., 2015).

2.4 Antibiotic Sensitivity Assay with Commercial Antibiotics

The disc diffusion method was employed for antibiotic sensitivity testing. Isolates were cultured in tryptone soya broth at 32°C, spread on tryptone soya agar plates, and incubated. Antibiotic discs (Oxytetracycline, Erythromycin, Ciprofloxacin, Chloramphenicol, Ampicillin, Furazolidone, and Gentamycin) were placed on the agar, and after incubation, zones of inhibition were measured to evaluate susceptibility or resistance (Shakerian et al., 2018).

III. RESULTS AND DISCUSSION

Total 100 fresh fish and shrimp samples were procured from selected aquaculture ponds and canals (each in triplicate) in various places of Nellore district were presented as follows (Table-5.1).

Isolation, phenotypic, and biochemical characterization of Vibrio isolates were performed following serial dilution, with samples cultured on TCBS agar plates at 32°C for 1 to 2 days. Ten suspected colonies were selected, leading to the identification of eight distinct isolates (VSP1–VSP8) based on Bergey's Manual (8th edition, 1974). The characteristics of these isolates, including variations in colony color and biochemical test results, are summarized in Tables 5.2 and 5.3. Specifically, VSP1 was identified as *Vibrio alginolyticus*, VSP2 as *Vibrio mimicus*, VSP3 as *Vibrio fluvialis*, VSP4 as *Vibrio harveyi*, VSP5 as *Vibrio splendidus*, VSP6 as *Vibrio vulnificus*, VSP7 as *Vibrio cholerae*, and VSP8 as *Vibrio parahaemolyticus*.

3.1 Antibiogram of the *Vibrio* isolates

The sensitivity of eight Vibrio species—*Vibrio alginolyticus* (Va), *Vibrio mimicus* (Vm), *Vibrio fluvialis* (Vf), *Vibrio harveyi* (Vh), *Vibrio splendidus* (Vs), *Vibrio vulnificus* (Vv), *Vibrio cholerae* (Vc), and *Vibrio parahaemolyticus* (Vp)—was evaluated against various commercial antibiotics, with results indicating susceptibility (S) and resistance (R) patterns (Table 5.4). Oxy tetracycline (OTC) was effective against Va, Vm, Vh, and Vs, while showing resistance in Vf and Vv. Erythromycin (ERY) was ineffective against Va, Vm, and Vf but moderately effective against Vh, Vs, Vv, and Vc. Ciprofloxacin (CIP) displayed effectiveness against Va, Vm, and Vf, with some resistance in Vh and Vs. Chloramphenicol (CAP) proved effective across all tested species, while ampicillin (AMP) was only effective against Vf.

Additionally, furazolidone showed broad effectiveness across all species, while gentamycin (GE) was moderately effective against Vm, Vh, Vs, and Vv, with resistance noted in Va, Vf, and Vc. These results underscore the varying susceptibility of Vibrio species to different antibiotics, highlighting the importance of ongoing surveillance for effective treatment options.

Table- 5.1 Fish and Shrimp sample collection from Various locations

Name of the area	Name of village	Name of the sample	No of samples	Length of sample (cm)	Weight of the sample(g)
Indukurpet	Gangapatnam, Somarajupalli, Nidimusali	*L.vannamei*	5	10 ± 2.2	9.5± 1.2
		Oreochromis mossambicus	5	15.3 ± 3.2	500 ±50.7
		Pangasius pangasius	5	28.9 ± 3.1	700 ±75.9
		Labeo rohita	5	24.9 ± 3.1	500 ±45.9
		Pampus chinensis	5	25.7 ± 4.3	500± 80.7
		Channa striata	5	30.5 ±2.7	600± 70.5
TP gudur u	Venkannapalem, Amuluru, Eduru	*L.vannamei*	5	10 ± 12.9	12 ± 3.5
		Oreochromis mossambicus	5	31 ± 2.1	600 ±82.2
		Pangasius pangasius	5	24.9 ± 3.1	500 ±45.9
		Labeo rohita	5	28.9 ± 3.1	700 ±75.9
		Pampus chinensis	5	27.3 ±4.6	600± 10.5
		Channa striata	5	32.7 ±5.7	800± 80
Allur	Iskapalli, Gogulap	*L.vannamei*	5	10 ± 11.2	9.5 ± 3.2

	alli, Alluru	*Oreochromis mossambicus*	5	10 ± 12.9	32.7± 2.5
		Pangasius pangasius	5	25.7 ±3.7	700± 50.5
		Labeo rohita	5	28± 5.2	750± 55.7
		Pampus chinensis	5	24.6 ±6.3	500.6±5.
		Channa striata	5	30 ± 12.5	700 ± 1.5
Kandaleru creek	Tippaguntapalem, Pudipatri	*Oreochromis mossa*	5	14.2 ± 16.7	44.1 ± 3.7
	, Kolanakudur	*mbicus*			
Buckingham canal	Gangapatnam, Koruturu, Mypadu	*Oreochromis mossambicus*	5	14.7 ± 3.2	52 ± 4.3

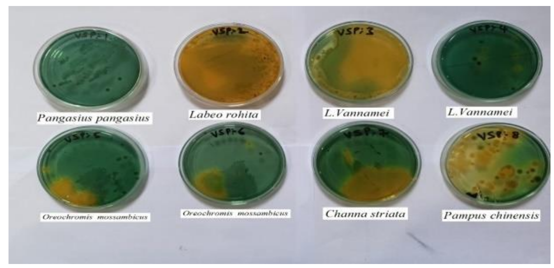

Fig-5. 1. Different Vibrio colonies on TCBS agar plate

Table-5.2 Characteristic colour of Vibio species grown in TCBS agar plates

S.No	Vibrio species	Extraction organ	Colour character
1	*V.alginolyticus* -Vsp1	Gut	Yellow
2	*V.mimicus* -Vsp2	Gut	Yellow
3	*V.fluvialis* -Vsp3	Gut	Yellow
4	*V.harveyi* -Vsp4	Gut	Green colonies
5	*V.splendidus* -Vsp5	Gut	Ggreen colonies
6	*V.vulnificus* -Vsp6	Gut	Green colonies
7	*V.cholerae* -Vsp7	Gut	Yellow
8	*V.parahaemolyticus-Vsp8*	Gut	Yellow

The study results provide a comprehensive examination of antibiotic resistance patterns among various Vibrio species isolated from fish and shrimp samples obtained from aquaculture ponds in the Nellore District. A total of 100 samples were analyzed, leading to the identification of eight distinct Vibrio species: *Vibrio alginolyticus* (Va), *Vibrio mimicus* (Vm), *Vibrio fluvialis* (Vf), *Vibrio harveyi* (Vh), *Vibrio splendidus* (Vs), *Vibrio vulnificus* (Vv), *Vibrio cholerae* (Vc), and *Vibrio parahaemolyticus* (Vp). The identification of these isolates was confirmed through biochemical characterization, including Gram staining, motility tests, colony morphology on TCBS agar, and additional biochemical assays. Key distinguishing factors included the ability of the isolates to utilize citrate and decarboxylate lysine, alongside pigmentation and motility (Srinivasan & Ramasamy, 2009).

Table-5.3 Morphological and Biochemical characterization of different *Vibrio* isolates

Test	VSP1	VSP2	VSP3	VSP4	VSP5	VSP6	VSP7	VSP8
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram Staining	-	-	-	-	-	-	-	-
Pigmentation in TCBS	Yellow	Yellow	Yellow	Green	Green	Green	Yellow	Yellow
Motility	+	+	+	+	+	+	+	-
Oxidase Test	+	+	+	+	+	+	+	+
Citrate Utilization	+	+	+	+	+	+	+	+
Voges-Proskauer Test	+	-	-	+	+	-	-	-
Halophilism test 0% NaCl	-	-	-	-	-	-	-	-
1% NaCl	+	+	+	-	-	+	-	+
3% NaCl	+	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+	+
8% NaCl	+	+	+	+	+	+	+	+
Halophilism 10%+ NaCl	+	+	+	+	+	+	+	+
Lysine Decarboxylase	+	+	+	+	+	+	+	+
Indole Production	+	+	+	+	+	+	-	+
Methyl-Red Test	+	+	-	+	+	+	+	-
Acid-from D-cellobiose	-	-	+	-	+	-	-	-
ONPG Test	+	+	+	+	+	+	-	+
Identification up to species	(Va)	(Vm)	(Vf)	(Vh)	(Vs)	(Vv)	(Vc)	(Vp)

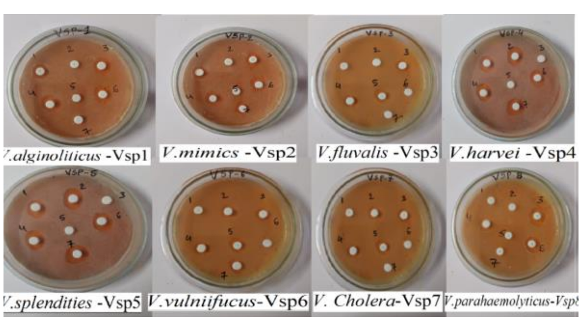

Fig-5.2 Antibiotic resistance and sensitivity pattern of vibrio isolates vsp1 to vsp 8 against 8 commercial antibiotics

The antibiotic sensitivity assays revealed concerning trends of resistance among the Vibrio species. For example, while *Vibrio alginolyticus*, *Vibrio mimicus*, *Vibrio harveyi*, and *Vibrio splendidus* exhibited susceptibility to oxytetracycline, with inhibition zones ranging from 2 to 6 mm, *Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* showed resistance. This raises alarms, as oxytetracycline is widely used in aquaculture. Erythromycin presented a mixed resistance profile, with resistance noted in Va, Vm, Vf, and Vp, while moderate effectiveness was observed against Vh, Vs, Vv, and Vc. The emergence of resistance to erythromycin, a critical antibiotic in both aquaculture and human medicine, raises concerns regarding the potential transfer of resistance genes from aquaculture to pathogenic bacteria (Jeyasanta et al., 2017).

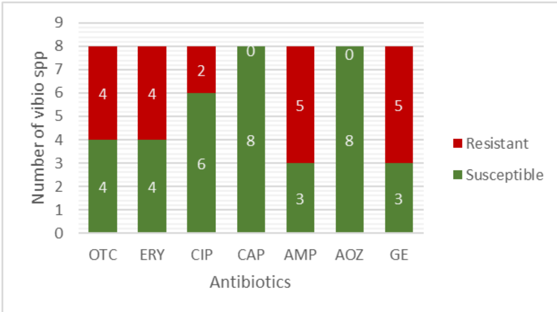

Fig-5.3 Vibro resistant and sensitivity patterns for the tested antibiotics

Table-5.4 Antibiotic sensitivity patterns of Vibrio isolates with commercial antibiotics (Zone of inhibition in mm)

S. No	Antibiotic	Conc./ Disc	Vibrio isolates (Zone of inhibition in mm)							
			Va	Vm	Vf	Vh	Vs	Vv	Vc	Vp
1	OTC	10mcg	2±0.3	6±0.4	0	5±0.2	4±0.4	0	0	0
2	ERY	15 mcg	0	0	0	6±0.4	4±0.3	4±0.2	4±0.4	0
3	CIP	10 mcg	4±0.2	4±0.3	4±0.2	0	0	2±0.4	3±0.5	2±0.7
4	CAP	10mcg	5±0.6	4±0.5	3±0.4	4±0.5	3±0.4	3±0.2	3±0.6	3±0.3
5	AMP	10mcg	0	0	5±0.3	0	0	3±0.2	3±0.5	0
6	AOZ	10mcg	3±0.7	5±0.3	3±0.4	5±0.5	5±0.4	4±0.6	4±0.7	5±0.5
7	GE	10mcg	0	5±0.3	0	5±0.6	0	3±0.6	0	0
0= Resistant										

Ciprofloxacin demonstrated effectiveness against Va, Vm, and Vf but showed resistance in Vh and Vs, with only moderate effectiveness against Vv, Vc, and Vp (Igbinosa et al., 2016). This indicates a troubling trend of ciprofloxacin resistance developing in aquaculture environments, potentially limiting its future effectiveness. Both chloramphenicol and furazolidone showed broad effectiveness across all tested Vibrio species, although the use of chloramphenicol is restricted in many regions due to its potential toxicity to humans (Silvester et al., 2019). Ampicillin demonstrated selective effectiveness, with only Vf showing susceptibility; resistance was prevalent among other species, which is particularly concerning given ampicillin's historical use in aquaculture (Devi et al., 2009).

Additionally, the response to gentamycin was varied, with significant resistance observed in strains such as Vv and Vc. This aligns with previous reports of increasing resistance to gentamycin in aquatic bacteria (Gupta et al., 2017). Overall, the findings underscore a growing issue of antibiotic resistance in aquaculture settings, particularly among Vibrio species. The rapid development of resistance, exacerbated by mechanisms such as horizontal gene transfer, highlights the urgent need for stricter regulations regarding antibiotic use in aquaculture. The study's data can inform future regulatory policies and disease management strategies, advocating for sustainable alternatives such as vaccines, probiotics, and enhanced biosecurity measures to mitigate reliance on antibiotics and preserve their effectiveness for both aquaculture and human health (Shakerian et al., 2018).

CONCLUSION

In this investigation, the antibiotic resistance patterns of *Vibrio spp* was found in shrimp and fish samples collected from aquaculture sites in the Nellore District of Andhra Pradesh and eight *Vibrio* species were identified. Varied antibiotic resistance patterns found during the study. Ciprofloxacin (CIP) was found to be most effective antibiotic, with six of the eight species exhibiting susceptibility. Significant resistance was noted against commonly used antibiotics like Oxytetracycline (OTC), Erythromycin (ERY), Ampicillin (AMP), and Gentamycin (GE), raising concerns about their efficacy. Chloramphenicol (CAP) and Furazolidone (AOZ) were highly effective, showing complete susceptibility in all species, yet their use is restricted due to safety regulations, especially in food production. The high resistance observed in AMP and GE emphasizes the need for more careful antibiotic selection and the exploration of alternatives like vaccines or probiotics.

REFERENCES

[1] Devi, R., Surendran, P. K., & Chakraborty, K. (2009). Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. *World Journal of Microbiology and Biotechnology*, 25(4), 541-549.

[2] Eid, H., Soliman, Z., & Hanafy, A. S. (2015). Prevalence and Molecular Characterization of Vibrio Spp. in Fish and Shellfish from Port Said Coastal Area. Suez Canal Veterinary Medical Journal. SCVMJ, 20(2), 291-302.

[3] Elmahdi, S., DaSilva, L. V., & Parveen, S. (2016). Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: a review. Food microbiology, 57, 128-134.

[4] Igbinosa, E. O. (2016). Detection and antimicrobial resistance of Vibrio isolates in aquaculture environments: Implications for public health. Microbial Drug Resistance, 22(3), 238–245.

[5] Jeyasanta, K. I., Lilly, T., & Patterson, J. (2017). Prevalence of *Vibrio* species in the cultured shrimp and their antibiotic resistance. *Asian Journal of Applied Science and Technology (AJAST)*, *1*(8), 100-111.

[6] Kumar, S., Lekshmi, M., Parvathi, A., Nayak, B. B., & Varela, M. F. (2016). Antibiotic resistance in seafood-borne pathogens. Foodborne pathogens and antibiotic resistance, 397-415.

[7] Raja, R. A., Sridhar, R., Balachandran, C., Palanisammi, A., Ramesh, S., & Nagarajan, K. (2017). Pathogenicity profile of Vibrio parahaemolyticus in farmed Pacific white shrimp, Penaeus vannamei. Fish & Shellfish Immunology, 67, 368-381.

[8] Shakerian, A., Barton, M. D., Akinbowale, O. L., & Khamesipour, F. (2018). Antimicrobial resistance profile and resistance genes of *Vibrio* species isolated from giant freshwater prawn (*Macrobrachium rosenbergii*) raised in Iran. *Journal of the Hellenic Veterinary Medical Society*, *68*(1), 79–88.

[9] Silvester, R., Pires, J., Van Boeckel, T. P., Madhavan, A., Meenakshikutti, A. B., & Hatha, M. (2019). Occurrence of β-lactam resistance genes and plasmid-mediated resistance among Vibrios isolated from southwest coast of India. *Microbial Drug Resistance*, *25*(11), 1-12.

[10] Srinivasan, P., & Ramasamy, P. (2009). Occurrence, distribution, and antibiotic resistance patterns of *Vibrio* species associated with viral diseased shrimp of South Indian aquaculture environment. *International Journal of Agriculture*, 1(2), 24-35.