



## Incidence of Vibriosis and Antibigram of isolates from shrimp culture ponds of Nellore coast. A.P

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### Abstract

*Bacterial diseases, mainly due to Vibrio spp. are responsible for high mortality and severe economic loss in aqua industry in developing countries. The incidence of high antibiotic resistance is reported from many hatcheries and culture ponds. The present study attempted to isolate Vibrios on TCBS agar and biochemically characterizes Vibrio species from culture ponds of the Nellore coast. A total of nine isolates were isolated among which five isolates were yellow and four isolates were green colored on TCBS agar. The Vibrio spp. isolated from shrimp culture systems of Nellore coast were tested for susceptibility to some commonly used antibiotics using the agar-disc diffusion method. The antibiotics tested include Ampicillin (10 mcg), Neomycin (30 mcg), Gentamycin (10 mcg), Erythromycin (15 mcg), Norfloxacin (10 mcg), and Tetracycline (10mcg), Penicillin G 2(10 mcg). The highest resistance was found to Gentamycin followed by Norfloxacin. Neomycin, Tetracyclin and Ampicillin. Only two isolates showed resistance to Erythrimycin and Penicillin.*

**Key words:** Vibriosis, Antibiotics, Antibiogram, Shrimp culture

### I. INTRODUCTION

India is the second largest shrimp producing country in the world after china, exporting to 127 countries with an export value of \$5.5billion. In India, there are 150 hatcheries and 100,000 shrimp farms occupying an estimated area of 140,000 hectares [1]. It possibly grow further with the increase in demand in export especially to United States, European Union, Middle East and China. On the other hand, shrimp production rate has declined in recent years because of the prevalence and spread of infectious diseases [2]. Vibriosis, disease predominantly caused by bacterial infection has been considered as the major cause of trade and industry loss in the aquaculture [3]. The distribution of Vibriosis is wide-reaching, caused by a range of Vibrio spp.[4] and they are the cause for high mortality and economic loss (tissue lesion or necrosis, body malformation, low growth) in shrimp industry in all the countries where shrimp culture is practiced [2, 5]. Investigations undertaken on diseases caused by Vibrio spp. in *Litopenaeus vannamei* from culture ponds of coastal Andhra Pradesh evidenced mainly the diseases like tail necrosis, shell disease, red disease, loose shell syndrome, and white gut disease. Among these, loose shell syndrome, white gut disease, and red disease caused mass mortalities in shrimp culture ponds. Some species of Vibrio namely *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio vulnificus*, and *Vibrio splendidus* are proposed to be associated with the diseased/infected shrimp. Though bacteria causing diseases in Penaeid shrimp are well thought-out to be opportunistic and manageable, the financial implication of losses due to mortality of shrimp or rejection of the infected shrimp is vast [2]. Not enough information is available on the incidence of infection of Vibrio sp. and management strategies of shrimp in tropical India. Such a kind of information is important to the farmers to be aware of the impact of the infection and to take feasible measures.

Currently, there are no universally acceptable pharmaceutical agents that are approved by the FDA for treating infections in shrimp aquaculture. The usage of some specific antibiotics like Erythromycin, Neomycin, Ampicillin, Gentamycin etc., is banned in shrimp culture. Hence, studies are in progress to improve disease control and treatment through other means. It is reported that antibiotics are used with feed or some other means to inhibit shrimp pathogens in rearing ponds. As the cultured shrimp is meant for human consumption, there are increasing concerns regarding the evolution or existence of antibiotic resistant bacteria in aquaculture. The present study was initiated to assess the occurrence and antibiotic resistance profile of *Vibrio* spp. in culture ponds of Nellore coast. Research on the occurrence of antibiotic-resistant bacteria in marine invertebrates which are intended for human consumption has its prominence. Considering the importance, this study aimed to determine the susceptibility pattern of antibacterial drugs against *Vibrios* isolated from the culture ponds of *Litopenaeus vannamei* shrimp in and around Nellore, Andhra Pradesh.

## II. MATERIALS AND METHODS

### 1.1. Sampling area

For the present study, 14 shrimp culture ponds were selected from various places (listed in *Table 1*) of Nellore coast. Sampling was done over a period of three months from February 2016 to April 2016. Total 14 samples were collected in sterile 250ml polypropylene bottles kept in ice box during transportation to the laboratory. Temperature, salinity and pH were recorded during sample collection.

### 2.2 Isolation and Enumeration of *Vibrio* species

After serial dilution, samples were inoculated on to Thiosulphate citrate bile salt sucrose (TCBS) agar plates for the enumeration of presumptive *Vibrio* counts (PVC) by spread plate technique. The plates were incubated at 32<sup>0</sup> C for 24hours and number of colony forming units was noted [6].

### 2.3 Bio-chemical characterization and identification of *Vibrio* isolates

The isolated colonies were biochemically characterized and identified with the help of Bergey's Manual of Determinative Bacteriology [7]. The standard biochemical and morphological tests like Colony Morphology, Gram staining, Motility test, Carbohydrates fermentation test, oxidase test, Vogus-Proskauer (VP) test, Decarboxylase reactions, Lysine decarboxylase test, ONPG tests [8] were used to characterise the isolated *Vibrios*.

### 2.4 Antibiotic sensitivity assay:

*Vibrio* spp. were grown in Tryptone soya liquid medium by incubating over night at 32°C. Each culture was spread on tryptone soya agar (Hi-media) plates and the antibiotic discs include Ampicillin (10 mcg), Neomycin (30 mcg), Gentamycin (10 mcg), Erythromycin (15 mcg), Norfloxacin (10 mcg), and Tetracycline (10mcg), Penicillin G 2(10 mcg) obtained from Hi-Media, Mumbai, India were placed on the agar surface. Inhibition zones were measured after 24 hours of incubation at 32°C [7].

## III. RESULTS AND DISCUSSION

### 3.1 Sample parameters

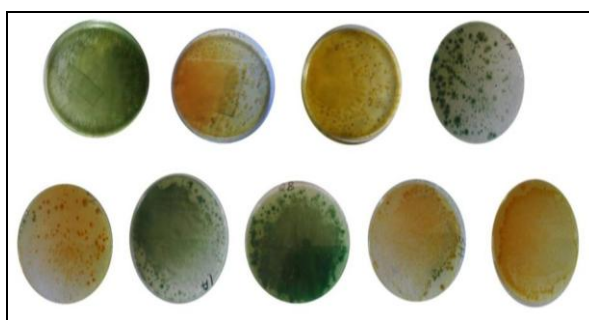
Total 42 water samples from the culture ponds from 14 places (each in triplicate) were collected and the parameters like Temperature, salinity and pH were recorded during sample collection and presented in *Table 1*.

*Table 1. Shrimp pond water sample collection*

S.No	Location/place	Salinity (ppt)	pH	Temperature (°C)
1	Indukurpalem	15	8.2	28
2	Iskapalli	16	8.0	30

3	Alicherla Bangarupalem	20	8.4	28
4	Pantapalem	15	7.8	29
5	Muthukuru	25	8.0	32
6	Pathapalem	15	8.0	32
7	Kothapalli	16	8.2	30
8	Koduru	15	8.2	30
9	Pathaputtapalem	16	7.9	30
10	Ganga patnam	20	7.5	32
11	Amuluvaripalem	15	8.0	32
12	Kotturu	12	7.5	30
13	Mypadu	15	8.0	32
14	Pallepadu	0	7.8	30

### 3.2 Isolation, phenotypic and Bio-chemical characterization



*Figure 1. Colonies on TCBS agar from the samples isolated from Indukur palem, Koduru, Bangaru palem, Iskapalli, Pallepadu, Kothapalli, Mypadu, Amuluvari palem and pallepadu respectively*

All the collected samples were subjected to serial dilution and transferred on to TCBS agar plates and incubated at 37 °C for 24 to 48 hours. 14 suspected colonies were selected from TCBS agar plates and finally nine different isolates (VSP1 to VSP9) were identified and confirmed based on the colony morphology, staining and biochemical tests. All the isolates belong to the genus *Vibrio* according to the limited description in Bergey's Manual of Determinative Bacteriology (8th edition, 1974). Results of morphological and biochemical tests are presented in Table 2.

*Table 2. Morphological and Biochemical characterization of Vibrio isolates*

Morphology and Biochemical test	VSP1	VSP2	VSP3	VSP4	VSP5	VSP6	VSP7	VSP8	VSP9
Gram stain	-	-	-	-	-	-	-	-	-
Growth on TCBS	Y	Y	G	Y	Y	G	G	G	Y
Motility	+	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+	+
Vogesproskauer test	+	-	+	+	+	+	-	-	-
Halophilism test									
0% NaCl	-	-	-	-	-	-	-	-	-
1% NaCl	+	+	+	-	+	-	+	+	-
3% NaCl	+	+	+	+	+	+	+	+	+
6% NaCl	+	+	+	+	+	+	+	+	+
8% NaCl	+	+	+	+	+	+	+	+	+
10% + NaCl	+	+	+	+	+	+	+	+	+

Lysine decarboxylase test	+	+	+	+	+	+	+	+	+
Methyl red test	+	+	+	+	-	-	+	-	-
ONPG test	+	+	+	+	+	+	+	+	+

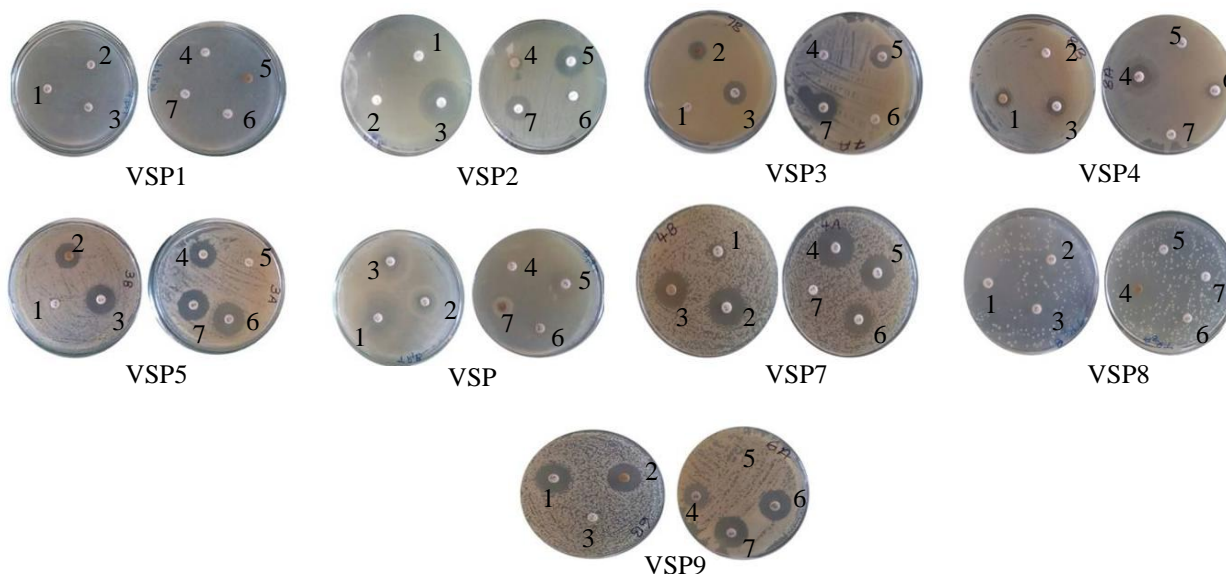
+ = positive, - = negative, Y = yellow, G = green

### 3.3 Antibiogram of the *Vibrio* isolates

In present study all the nine isolates were subjected to antibiotic susceptibility testing against seven commercial antibiotic disks. Among all the isolates, six isolates were resistant to Gentamycin, five isolates resistant to Norfloxacin, four isolates were resistant to Ampicillin, Tetracyclin and Neomycin, two isolates were resistant to Erythromycin and Penicillin. The results showed in Table-3 & Figure 2.

*Table 3. Antibiotic sensitivity patterns of Vibrio isolates: (Zone of inhibition in mm)*

Vibrio isolates	Ampicillin (10mcg)	Erythromycin (15mcg)	Penicillin G2 (10mcg)	Tetracycline (10mcg)	Neomycin (30mcg)	Gentamycin (10mcg)	Norfloxacin (10mcg)
Vsp1	-	9	-	-	14	-	-
Vsp2	-	-	16	-	16	-	14
Vsp3	-	14	14	-	14	-	10
Vsp4	8	-	14	14	-	-	-
Vsp5	-	16	12	17	-	14	16
Vsp6	10	14	16	-	14	-	-
Vsp 7	8	12	10	10	16	16	-
Vsp8	10	14	16	18	-	-	-
Vsp9	8	10	-	14	-	16	18



*Figure 2. Antibiogram of Vibrio isolates Vsp1 to Vsp 9*

(1= Ampicillin, 2= Erythromycin, 3= Penicillin, 4= Tetracycline, 5= Neomycin, 6= Gentamycin, 7= Norfloxacin)

#### IV. CONCLUSION

The present study shows that diseases caused by *Vibrio* spp. are quite prevalent in shrimp from culture ponds of coastal Andhra Pradesh, and the disease-induced morbidity/mortalities are causing considerable damage to the culture system. Special focused attention is therefore needed for controlling these diseases. In all the diseased shrimp subjected to the study, the bacterial flora comprised largely *Vibrio* species. With the increase of antibiotic use and due to many other environmental factors, marine *Vibrio* spp are gaining resistance to valuable antibiotics like ampicillin, penicillin, and erythromycin. In order to minimise the use of antibiotics, application of probiotics is suggested in both grow-out culture ponds and hatcheries. Application of probiotics and prebiotics as immunostimulants is an interesting prospect for replacement of antibiotics in the aquaculture industry and could be a useful tool in the rearing of delicate early stages of certain marine species.

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