Isolation and identification of *Vibrio* species from *Macrobrachium rosenbergii* cultured in selected five reservoirs in Uva and Southern provinces in Sri Lanka

L.N.M.P. Jayasinghe[1], S.U. Pathiranage[2], E.G.K.Y.C. Bandara[1], H.H.S. Pramodhi[1], K.V.D.M. Hasintha[2], H.C. Nadishani[2], D.N.N. Madushanka[2], K.H.M.A. Deepananda[1], H.A.D. Ruwandeepika[2*]

[1] *Department of Aquaculture, Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Wellamadama, Matara, Sri Lanka*

[2] *Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, P.O. Box 02, Belihuloya, Sri Lanka*

[*] *ruwandeepika@yahoo.co.uk*

1. Introduction

Macrobrachium rosenbergii (Giant Freshwater Prawn/GFP), residents in the tropical freshwaters adjacent to brackish water areas are more popular among the culture of crustaceans due to their rich taste and profitability. *M. rosenbergii* culture is now an emerging sector in Culture-Based Fishery in perennial and non-perennial reservoirs, Sri Lanka. Though it is profitable business, at present, *M. rosenbergii* production in Sri Lanka is low in both quality and quantity. Most devastating threat is the presence of disease-causing agents and among the diseases causing agent, microbes play an important role. Bacteria, especially the *vibrios* have a significant impact on *M. rosenbergii*.

Most of the studies have identified disease causative bacteria on GFP with frequently encountered *Vibrio* species. *Vibrios* are Gram-negative, rod-shaped, facultative anaerobes, (family Vibrionaceae), abundant in warm, brackish water and they lead to the devastating disease; *vibrio*sis. Even though there are non-pathogenic *vibrios*, about 12 species cause infections in humans by consumption of *Vibrio* contaminated seafood, water, and by wound exposing to water containing *Vibrios*, hence the zoonotic *vibrio*sis. Among the diverse disease out come in aquaculture species by *vibrios*, luminous *vibrio*sis, fouling disease, blackening of gills, brown spots and white muscle disease are some significant infections in prawn culture.

Though there are reports at global level, there is hardly any report on the *vibrios* associated with *Macrobrachium rosenbergii* in Sri Lankan reservoirs. Hence this study aimed to enumerate total bacteria and *vibrios* present in *Macrobrachium rosenbergii;* collected from five reservoirs, in Uva and Sothern provinces and also to isolate and identify *Vibrio* species associated with *M. rosenbergi.* Further studied the sensitivity of the *Vibrio* isolates to commonly used antibiotics.

2. Materials and Methods

2.1 Sample collection

Seventeen prawn samples were collected from three reservoirs (Urusita wewa, Muthukandiya, Handapanagala wewa) from Uva Province and two reservoirs (Ridiyagama, Bandagiriya) in Southern Province.

2.2 Sample preparation

Prawn samples were weighed, washed and homogenized in a stomacher (BagMixer 400cc, France). Homogenized samples were used for further study, enumeration of total bacteria and *vibrios*. Also, the samples were used for the isolation of *vibrios* as described in the Isolation and Identifications (2.4)

2.3 Enumeration of total bacteria and *Vibrios*

Enumeration of total bacteria and *Vibrios* present in the *M. rosenbergi* was done by using the spread plate method. Samples were serially diluted in sterilized distilled water and cells were enumerated by using the spread plate method using Plate Count Agar (Total Bacterial count), and in TCBS Agar (Vibrio yellow and green colony count). Colonies were enumerated (automated colony counter; as Colony Forming Units per gram/CFU/g).

2.4 Isolation and identification

Homogenized samples were enriched (APW); streaked in TCBS plates. Selected green and yellow colonies from TCBS were grown in LB broth. For identification of *vibrios*, battery of biochemical tests was done using Alsina`s key (1994) and Food and Drug Administration (FDA) bacterial analytical manual (BAM) as the table below.

Table 02. Biochemical tests

Test	Result
Motility test	Cloudy growth from stabbed line
Salt tolerance(0%,2%,6%,8%NaCl) Growth at 4°C,35°C,40°C	Cloudy growth
Amino acid decarboxylation	Positive- Purple colour
Indole test	Positive-Pink ring
Citrate test	Positive – Blue colour
Oxidase test	Positive- Blue colour
Catalase test	Positive- air bubbles
ONPG test	Positive- yellow colour
TSIA slant test	Positive- yellow butt Negative- orange/red butt
Caseinase test Gelatinase test	Positive-clear zones
VP test	Positive-Red layer
Urease test	Positive- Magenta
Oxidation/Fermentation test	Positive-Yellow colour
Grams staining	Gram negative

2.5 Antibiotic Susceptibility Test (ABST)

Antibiotic sensitivity test was performed by using the Kirby Bauer disk diffusion method (Bauer et al., 1966). ABST was performed for 9 Vibrio isolates with five commonly used antibiotics viz, Chloramphenicol (C30), Ciprofloxacin (CIP30), Tetracycline (TE30), Gentamicin (GEN30), and Ampicillin (AMP10) (Himedia, India). Diameters of inhibitory zones were compared with a standard chart that shows the performance standards (CLSI 2017).

3. Results and Discussion

Enumeration of Total Bacteria and *Vibrios*

The highest number of total bacterial count was present in Bandagiriya (9.32 ± 2.2 log CFU/g of prawn) whereas the Handapanagala reservoir has the lowest (7.02 ± 0.14 log CFU/g of prawn). The total number of *Vibrio* (yellow) colony counts was greater in every reservoir than Vibrio (green) colony count and the highest number of total Vibrio (yellow) colony counts was resulted in Bandagiriya wewa (6.64 ± 0.63 log CFU/g of prawn) while the lowest number was resulted in Muthukandiya (4.5 ± 1.31 log CFU/g of prawn). The highest number of total Vibrio (green) colony counts were in Bandagiriya (5.72 ± 0.73 log CFU/g of prawn) whereas the Muthukandiya wewa has the lowest (3.86 ± 0 log CFU/g of prawn) (Figure 4).

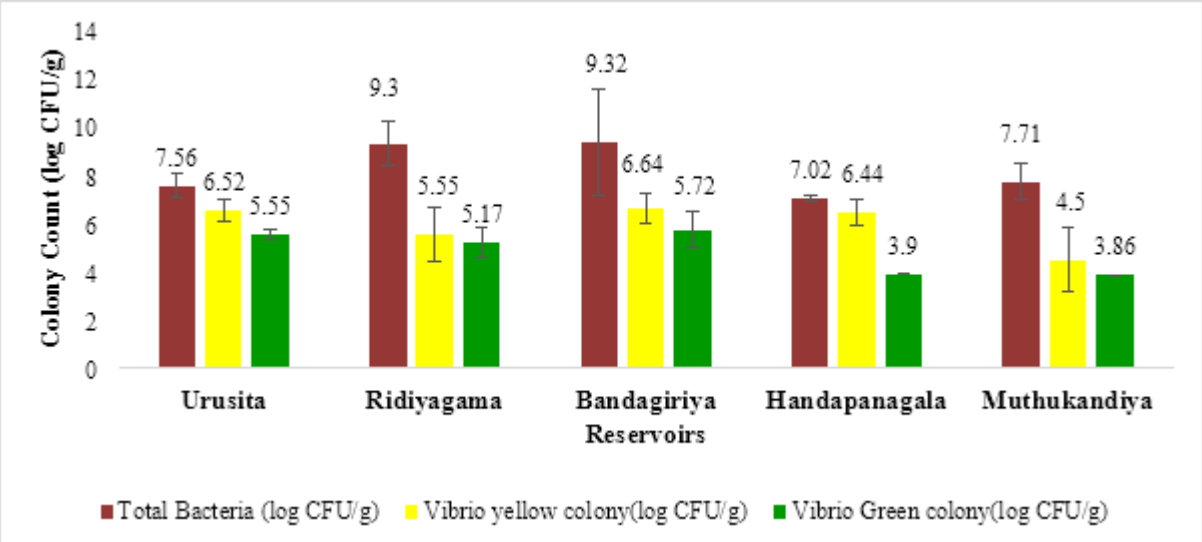

Figure 4. Enumeration of bacteria and *Vibrios* in *M. rosenbergii* collected from five reservoirs

A study done by Phatarpekar et al. (2002) is in line with the current study having a different distribution of bacteria and the *vibrios* among the different locations. Liu et al. (2020), concluded that there is an effect of the surrounding environment on microbial colonization in *M. rosenbergii* strongly agreed with us as we also had different colonization from different locations.

Forty-one isolates were identified as *Vibrios* and among them, 13 isolates were identified up to species level; *Vibrio parahaemolyticus, V. anguillarum, V. cholera, V. vulnificus, V. mimicus, V. alginolyticus, V.damsela, V. gazogenes, V. natriegens, V. metschnikovii.*

The species identification of *M. rosenbergii* done by Prakash and Karmagam (2013), has used TCBS to isolate *Vibrios* and a battery of tests as we used. As in the current study, *Vibrio alginolyticus, Vibrio cholera, Vibrio mimicus,* and some other *Vibrios* have identified in a study in Vietnam by Oanh et al. (2001). Different strains of *V. cholera* cause negative effects on hepatopancreas (Gao et al., 2019) and cause White Faeces Disease (WFD) in prawns. Disease investigation caused by *Vibrio alginolyticus* has recorded histological alterations in hepatopancreas, gills, muscle, and heart and lower the quality of prawns causing cloudy musculature, loss of appendages, and focal necrosis of the hepatopancreas (Ajadi et al., 2019).

All the identified isolates were resistant to Ampicillin 10µg while all the isolates were sensitive for Ciprofloxacin 30µg except *Vibrio cholera*. *V.damsela*, and *V.cholera* were resistant against Chloramphenicol 30µg where the others were sensitive. Only *V.anguillarum* and *V.cholera*

were shown a resistivity for Tetracycline 30µg. For Gentamicin, *V.anguillarum, V.alginolyticus,* and *V.gazogenes* were sensitive, while *V.mimicus, V.damsela, V.metschnikovii, V.vulnificus,* and *V.natriegens* were intermediate resistant and only *Vibrio cholera* was resistant. Overall, *Vibrio cholera* was resistant to all five antibiotics (Table 2).

Table 02. Antibiotic sensitivity of *Vibrio* spp isolated from *M. rosenbergii*

	Antibiotics Inhibition zone diameter in mm				
Vibrio isolate	CIP (30µg)	GEN (30µg)	TE (30µg)	C (30µg)	AMP (10µg)
Vibrio anguillarum	SEN	SEN	RES	SEN	RES
Vibrio mimicus	SEN	IR	SEN	SEN	RES
Vibrio damsela	SEN	IR	SEN	RES	RES
Vibrio cholera	RES	RES	RES	RES	RES
Vibrio metschnikovii	SEN	IR	SEN	SEN	RES
Vibrio vulnificus	SEN	IR	SEN	SEN	RES
Vibrio gazogenes	SEN	SEN	SEN	SEN	RES
Vibrio alginolyticus	SEN	SEN	SEN	SEN	RES
Vibrio natriegens	SEN	IR	SEN	SEN	RES

CIP: Ciprofloxacin, GEN: Gentamicin, TE: Tetracycline, C: Chloramphenicol, AMP: Ampicillin.
SEN=Sensitive,RES=Resistant,IR=Intermediate resistant

There are several studies showing antibiotic resistance in line with findings of the current study. Amalina et al, 2019 reported the resistant of *vibrios* isolated from groupers to Ampicillin while all of them were sensitive to Tetracycline. Rahman et al, 2020 revealed that *vibrios* isolated from *Penaeus monodon* were sensitive for Ciprofloxacin, Chloramphenicol, and Tetracycline. There are ample of reports showing different sensitivities by *Vibrio* spp isolated from aquatic animals.

4. Conclusions

This study concluded that there is a variation of total bacteria and *Vibrios* present in *M. rosenbergii* and there is a diversity among identified *Vibrios*. Moreover, it concluded that there is a resistance development by *Vibrios* isolated from *M. rosenbergii* to commonly used antibiotics, this is an alarming message derived from this study.

5. References

Alsina, M., & Blanch, A. R. (1994). A set of keys for biochemical identification of environmental *Vibrio* species. *Journal of applied bacteriology*, *76*(1), 79-85.

Amalina, N. Z., Santha, S., Zulperi, D., Amal, M. N. A., Yusof, M. T., Zamri-Saad, M., & Ina-Salwany, M. Y. (2019). Prevalence, antimicrobial susceptibility and plasmid profiling of *Vibrio* spp. isolated from cultured groupers in Peninsular Malaysia. *BMC microbiology*, *19*(1), 1-15.

Ajadi, A., Sabri, M. Y., Atata, J. A., Daodu, O. B., & Emikpe, B. O. (2019). Pathology and immunohistochemical evaluation of Vibrio alginolyticus infection in *Macrobrachium rosenbergii*. *Comparative Clinical Pathology*, *28*(2), 359-368.

Gao, X., Miao, Z., Li, X., Chen, N., Gu, W., Liu, X., & Zhang, X. (2019). Pathogenicity of non-O1/O139 Vibrio cholerae and its induced immune response in *Macrobrachium rosenbergii*. *Fish & shellfish immunology*, *92*, 300-307.

Liu, B., Zhou, Q., Sun, C., Song, C., Zhang, H., Yang, Z., & Shan, F. (2020). Patterns of bacterial community composition and diversity following the embryonic development stages of *Macrobrachium rosenbergii*. *Aquaculture Reports*, *17*, 100372.