International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 1 Number 1 (2013) pp. 01-16

www.journals.excellentpublishers.com

A study on bacterial flora associated with fresh water prawn, *Macrobrachium rosenbergii*

M.Prakash[1*] and N.Karmagam[2]

[1]Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi-631 551, Kancheepuram, Tamil Nadu, India
[2]Department of Botany, Govt. Arts College, Salem, Tamil Nadu, India.

***Corresponding author e-mail:** *mpraksahmicro@gmail.com*

KEYWORDS

Giant freshwater prawn; *Macrobrachium rosenbergii*; Total coliform Count; Total Bacterial Count; Proteolytic activity; lipolytic activity; antibiotic sensitivity.

A B S T R A C T

The giant freshwater prawn *Macrobrachium rosenbergii*, popularly known as scampi, farmed crustacean species was used in this study. Fresh water prawn samples collected in two places. First place, Fresh water Prawn hatchery area, Marakanam and Neelakarai, this sample should be considered as a set=1. Second place, samples from various market areas in and around Kancheepuram town, Tamilnadu, this sample should be considered as set=2. The correlation between Total coliform Count (TCC) and Total Bacterial Count (TBC) of Set 1 and 2 was considerably significant statistically ($r=0.40; p<0.01$ and $r=0.55; p<0.01$). The positive regression line in the present study also supports that the increase in TCC with the increase in TBC. The principal bacterial genera encountered in fresh water prawn comprised of *E. coli, Pseudomonas sps, Enterobacter sps, Vibrio sps Aeromonas sps and Staphylococcus aureus.* Proteolytic activity and lipolytic activity were also found in the bacterial isolates which were isolated from giant fresh water prawn (*Macrobrachium rosenbergii*) digestive system. The antibiotic sensitivity showed Oxytetracycline antibiotic was highly sensitive to most of the bacterial isolates from the prawn samples. Hence, Knowledge of the qualitative and quantitative aspects of bacterial flora in the hatchery would help to understand disturbances, if any, brought about during disease outbreaks.

Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii (*de Man, 1879) (or scampi) is an important commercial species due to property as food supply as well as a valuable export product. In India, giant freshwater prawn distributes mainly in the Southern region where environmental conditions are most favorable for the

growth of scampi. Increasing demand of this species for domestic consumption and export markets has increased remarkably scampi cultured systems with large scale, high stocking density and intensive feeding. Consequently, cultivation of this economic species is being expanded to culture in rice fields, orchard gardens, pens along river banks. Hence, disease is inevitable in these uncontrollable culture models.

In addition, the use of antibiotics to control bacteria population and maintain healthy environment for prawn culture becomes popular. A wide range of antibiotics is now being used to treat bacterial diseases and to control bacterial population in the hatcheries and prawn farms. The potential consequences of used antibiotics for treatment may arise various antibiotic resistant, antibiotic-resistant bacteria. The phenomenon resistance was transfer to pathogenic bacteria, and led to reduce efficacy of antibiotic treatment for disease caused by the resistant pathogens (Frappaolo *et al*., 1986).

The growth of the shrimp aquaculture industry increased the need to intensify farming practices to maximize profits. Problems of diseases often accompanied this intensification as environmental conditions deteriorated and brought the decline of the industry. Pressure to ensure production led to reliance on antibiotics. Chemotherapy is widely practiced in the Philippines Baticados and Paclibare, (1990). Antibiotics are administered to farmed shrimp primarily to prevent or treat bacterial diseases. In Philippine grow-out ponds, oxytetracycline, oxolinic acid, chloramphenical and furazolidone are incorporated in artificial feeds as treatment against luminescent vibriosis. Other antibacterial agents used in intensive prawn farms in the Philippines include the nitrofurans, erythromycin, and sulfa drugs Baticados *et al*., (1990) and Primavera, *et al*., (1993).

The presence of antimicrobial agents at low concentration through leaching or continued usage may lead to the development of drug-resistant strains and multiple antibiotic resistance MAR. In bacteria which may result to resistance transfer to pathogenic bacteria and reduced efficacy of antibiotic treatment for human and animal diseases. Several studies have been done to investigate the possible consequences of the use of antimicrobials. Most of the studies are focused on fish and its environment. Spanggaard *et al*., (1993) reported resistance to oxytetracycline among bacteria from freshwater fish farms in Denmark. Nygaard *et al*, (1992) reported that exposure to oxytetracycline and oxolinic acid initiates resistance to other drugs. McPhearson *et al*, (1991) observed that individual and multiple antibiotic resistances were associated with antimicrobial use. Antibacterial-resistant bacteria in sacrificial sediments near salmon net cage farms were isolated by Herwig *et al*, (1997) suggesting that these antimicrobials may increase the level of resistance in bacteria in the surrounding environments. According to Kerry *et al*, (1995) resistant flora in feed can, under certain circumstances, significantly contribute to the resistant flora detected in sediments under fish cages. Kerry *et al*, (1997) on the other hand, reported that emergence of strains resistant to oxytetracycline in the microflora of the intestine of Atlantic Salmon held in seawater is not a necessary consequence of its oral administration.

A wide range of antimicrobial compounds (oxytetracycline, ciprofloxacin, nitrofurantoin, furazolidone or

chloramphenicol) is now being used in the hatcheries and farms of freshwater prawn and marine shrimp in India to control bacterial population (Karunasagar *et al*., 1994; Abraham *et al*., 1997; Sahul Hameed and Balasubramanian, 2000). The potential consequences of antibiotic use in the treatments are the development of antibiotic-resistant microorganisms, multiple antibiotic resistance, resistance transfer to pathogenic bacteria, and reduced efficacy of antibiotic treatment for diseases caused by resistant pathogens (Frappaolo and Guest, 1986).

Fresh water prawn culture is rapidly expanding and *Macrobrachium rosenbergii* is an important species cultured in many countries. Production of healthy and quality seeds has been a major obstacle in the expansion of the culture of *M.rosenbergii*. A complex mix of environmental factors, microbiological profiles and management practices influence the success of the production cycle. Microorganisms have been implicated in many disease conditions such as bacterial necrosis (Aquacop, 1977) and larval mid-cycle disease (MCD) (Brock, 1983). Anderson *et al*., (1989) identified *Alcaligenes* spp. and *Enterobacter* spp. from larvae with MCD. The occurrence of viral (Anderson *et al*., 1990; Tung *et al*., 1999) and fungal diseases (Anderson, 1988; New, 1995) has been reported.

Luminescent bacterial disease due to Vibrio spp. (Tonguthai, 1995) has been reported to cause serious mortalities in *M.rosenbergii* hatcheries. Lavilla-Pitogo *et al*., (1990) and Karunasagar *et al*., (1994) reported luminescent vibriosis to be a major problem causing significant mortalities in systems employing saline waters such as Penaeus monodon hatcheries. The larval rearing practices favour the rapid multiplication of bacteria in the system. However, most bacteria are part of the commensal flora and only some of them may be opportunistic pathogens. There are not many reports regarding the normal bacterial flora associated with *M.rosenbergii* hatcheries. Miyamoto *et al*., (1983) identified 13 genera of bacteria from *M.rosenbergii* larvae, including *Aeromonas*, Pseudomonas and Vibrio as the dominant flora while Anderson *et al*., (1989) studied the aerobic heterotrophic microflora in *M.rosenbergii* hatcheries in Malaysia and reported *Alcaligenes* spp. and *Vibrio* spp. to be the most frequently encountered genera. Colorni (1985) investigated the role of Artemia as feed in determining the health of *M.rosenbergii* and the possibility of bacterial pathogens being introduced into the system through Artemia. Anderson *et al*., (1990) showed the significance of hygiene and sanitary practices in precipitating larval mortalities in *M.rosenbergii* reared in modified “green water” system in Malaysia. The Green water system consists of culturing simultaneously both phytoplankton and zooplankton in the same tank in which the larvae are reared simulating a natural ecology (Fujinaga, 1969), while the ‘Clear water or Galveston system’ of larval rearing consists of producing algae and zooplankton which are used as feed for the larvae in units which are completely separate from the tanks where the larvae are reared. In the Clear water system the stages of larvae are typically reared in different containers (Barnabe, 1990; Smith *et al*., 1976).

Vibriosis is a major disease problem in shrimp aquaculture, causing high mortalities and severe economic losses in all countries (Brock and Lea Master, 1992; Lightner, 1988; Mohney *et al*., 1994). It is most often considered as an opportunistic

pathology in shrimp, but a primary disease caused by highly virulent strains of *Vibrio* sp. has also been reported De la Pena *et al*., 1993. The major genospecies causing vibriosis in shrimp are *Vibrio harĺeyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. anguillarum (*Lightner, 1988; Lightner, 1996; Jiravanichpaisal *et al*., 1994). These pathologies occurs at all rearing levels, from hatchery tanks to grow out ponds. However, specificity has been reported both at the species and the stage level, so strains responsible for larval and juvenile vibriosis are considered to be different, even when belonging to the same species.

To study the bacterial flora from the digestive system of fresh water prawn, (*Macrobrachium rosenbergii*).To enumerate the total bacteria count and total Coliform count in the digestive system of fresh water prawn.To isolate and identify the bacterial flora from the digestive system of fresh water prawn (*Macrobrachium rosenbergii*).To identify the proteolytic activity and lipolytic activity from the bacterial isolates. To identify their susceptibility to various antibacterial agents to know the effective therapeutic agent as well as to know the resistant pattern.

Materials and Methods

Freshwater prawn

The giant freshwater prawn *Macrobrachium rosenbergii*, popularly known as scampi, farmed crustacean species was used in this study. Fresh water prawn samples collected in two places. First place, Fresh water Prawn hatchery area, Marakanam and Neelakarai, this sample should be considered as a set=1. Second place, samples from various market areas in and around Kancheepuram town, Tamilnadu, this sample should be considered as set=2. These samples were collected and adopting precautionary methods for transported to the microbiological laboratory. In this study about 100 samples were collected i.e., set=1, 60 samples and set=2, 40 samples and processed.

Collection

Bacteriological examination of any food product depends upon the proper collection, transportation to the laboratory and preparation for examination care should be taken to fulfill those requirements. Prawn is a perishable food so it should be collected and refrigerated preferably at 0°C to 4°C with the help of an ice box and as a general rule samples should be examined with in 36 hours after sampling.

Dissection of Prawn

Uniformly, for all samples were surface disinfected with 25 ppm sodium hypochlorite for 10 minutes before dissecting out the alimentary canal. Prawn gut was opened by using sterile scalpel, forceps and sterile knife. The alimentary canal was divided in to stomach (Proventriculus), anterior intestine (Anterior midgut) and posterior intestine (Posterior midgut along with the hind gut). Each gut region contents squeezed out in to sterile weighing bottles separately.

Enumeration of Total Bacterial Population

The total bacterial densities were enumerated employing spread plate method. 1 gm of each dissected gut contents were added into separate flasks containing 100 ml of sterile distilled water blanks. Further serial dilutions were made using 9ml sterile distilled blanks. The

samples were serially diluted to 10^5 for the estimation of total plate count for bacteria.
The serially diluted samples were processed to estimate the Total Bacterial Count (TBC) and Total Coliform count (TCC) using spread plate technique. The samples were serially diluted and plated on Tryptic Soya Agar (TSA). TCC was determined by plating on Violet Red Bile Agar(VRB). The plates were incubated at 30 °C±2 °C for 24–48 h. Plates with 30–300 colonies were taken to determine the counts. Representative colonies were picked for further identification.

Bacteriological Techniques for identification

The isolates were inoculated over Nutrient Agar, Blood Agar and MacConkey agar. With the exception of the possible presence of Vibrio species TCBS agar are also inoculated and the entire above are incubated at 37°C for 24 hours.

Identification of bacteria

Isolated colonies were purified and pure cultures were used for biochemical tests (MacFaddin, 1980). After noting the morphology and pigmentation of the colony, the morphologically dissimilar colonies were randomly selected and the pure culture was maintained on nutrient agar and stored at 4˚C for identification of the isolates. The organism grown over the above medium was analyzed for their morphological, cultural and biochemical characters. The generic composition of the bacterial strains were identified by following the methods of Shewan *et al* (1971); Gilmour *et al* (1975), Bergy's Manual of Determinative Bacateriology (1984,1989).

Gram-positive bacteria were identified up to generic level following the scheme of Le Chevallier *et al*. (1980) and for Gram-negative bacteria the scheme of Bain and Shewan (1971) and LeChevallier *et al*. (1980) was used. Vibrio spp. was identified following the scheme of Farmer and Hickman-Brenner (1992).

Lipolytic activity

Lipids are high molecular components, that processing large amount of energy. The degradation such as by triglyceride accomplished by extracellular hydrolytic enzyme called lipases that cleave the ester bonds in their moles by the addition and H_2O to form the building blocks glycerol some of othe microorganisms have the ability the hydrolyse lipids. Tributyrin agar is used to demonstrate the hydrolytic activity of exo enzyme lipase. Triglyceride tributyrin forms an emulsion when disperse in the agar producing an opaque medium for observing exo enzymatic activity. Tributyrin medium was prepared with 0.5% peptone, 0.3% yeast extract, 2% agar, and 1% tributyrin. The media were sterilized by autoclaving at 131°C for 15 mins. Lipase-positive microorganisms were detected by their ability to hydrolyze the lipid substrates to produce free fatty acids. Colonies of lipolytic microorganism's tributyrin medium surrounded by zones against a turbid background of emulsified, unhydrolyzed lipid. (Bright Sigh, *et al*, 1998)

Statistical analysis

Correlation and regression analysis were calculated by using SPSS 9.0 packages. Correlation analysis was used to detect any relationship between the Total bacterial count and Total Coliform count.

Antimicrobial sensitivity test

Antibiotic sensitivity test was performed in Muller Hinton agar medium. A lawn culture was prepared on the media with the swab from the culture in nutrient broth. Different antibiotic discs were placed on the media using sterile forceps. After 24 hours of incubation the clear zone of inhibition around the disc was measured and the results were noted (modified kirbey – Bauer technique, 1966).

Result and Discussion

A total of 100 fresh water samples were collected for the study from Marakanam and Neelakarai (set=1; n=60) and different market places (set=2;n=40) of Kanchipuram town. The first set of prawn samples (Set 1, n=60) showed a TBC range between $8x10^3$ and $145x10^3$ CFU/ml, TCC range between $4.3x10^3$ and $5.8x10^3$ CFU/ml. The relative index TCC/TBC was p_i = 0.17. The calculated regression line was *y=0.5758x+2.414* for TCC vs TBC. The correlation between TCC and TBC was highly significant (*r=0.40; p<0.01*) (Chart.1).

In Set 2 (n=40), TBC ranged between $98.7x10^3$ and $146.5x10^3$ CFU/ml. TCC ranged between $0.4x10^3$ and $55.0x10^3$ CFU/ml. The regression lines for TCCxTBC were defined by the equations *y=0.561x+2.365* (Chart.2). The relative index TCC/TBC was p_i = 0.27. The correlation between TCC and TBC was highly significant (*r=0.55; p<0.01*) (Table.1) (Chart.2). Results on analysis of phenotypic characters of bacterial isolates were shown in Table-2.

There were 6 bacteria species identified from 100 (set=1 and set=2) fresh water prawn samples. Results on percentage of bacterial isolates obtained from the set=1 and set=2 fresh water prawn were shown in Table.4. Results on Proteolytic activity and lipolytic activity of bacterial isolates obtained from the fresh water prawn were shown in Table.5 and Table.6.

The bacteriological examination of different places of prawn samples grouped into Set 1 and 2 showed surprisingly higher counts of total bacteria, and coliform bacteria. The correlation between TCC and TBC of Set 1 and 2 was considerably significant statistically (r=*0.40; p<0.01* and *r=0.55; p<0.01*) respectively. The positive regression line in the present study (Chart 1 and 2) also supports that the increase in TCC with the increase in TBC. The report of Bob Oxley *et al*, 2002 and Kennedy, *et al* 2006 fall in line with the present study findings that they also observed the increase of coliforms when the total bacterial count increased in digestive tract of prawn samples. Anderson *et al*., (1989); Bright Sigh, *et al*, (1992) observed that only a slight correlation between TCC and total bacterial counts of prawn samples whereas Chen, *et al* (1990) and Phatarpekar *et al* (2002) observed high correlation between TCC and TBC and it might imply that TBC alone is a significant indicator of hygienic quality of prawn samples.

In the present study, bacterial flora observed on the digestive system of fresh water prawn reared and market prawn, *Macrobrachium rosenbergii* showed Escherichia coli as the prominent bacteria, a reflection of the bacterial flora of the water as suggested by Roberts, (1978); Vanderzant *et al* (1970) reported Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter and Aeromonas, and Vibrio sps as the dominant flora with

Chart.1 Enumeration of Total bacterial count and total Vibrio count in digestive system of Fresh water prawn *Macrobrachium rosenbergii*. (Set=1; n=60)

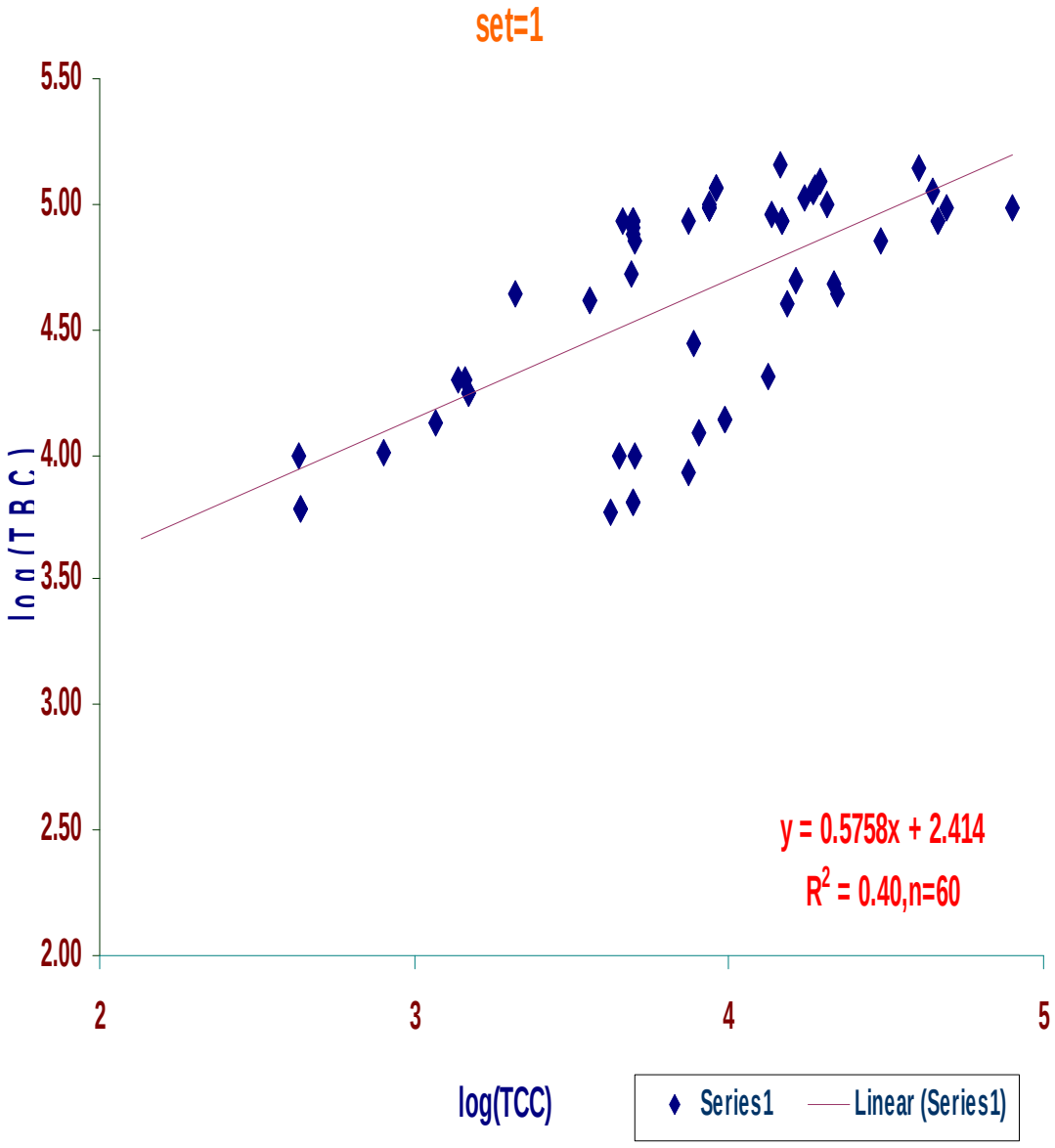

Table.1 Enumeration of Total bacterial count and total Vibrio count in digestive system of Fresh water prawn *Macrobrachium rosenbergii*. (Set=2; n=60)

SET=2				
Sample No.	**TCC**	**TBC**	**TCC (log)**	**TBC(log)**
1	3638	49672	3.56	4.70
2	477	6000	2.68	3.78
3	14712	17480	4.17	4.24
4	38750	106000	4.59	5.03
5	98700	134972	4.99	5.13
6	4150	48000	3.62	4.68
7	3600	44220	3.56	4.65
8	19600	144500	4.29	5.16
9	16781	131240	4.22	5.12
10	5400	9800	3.73	3.99
11	1360	22000	3.13	4.34
12	29780	80477	4.47	4.91
13	2101	24000	3.32	4.38
14	540	9700	2.73	3.99
15	1340	20400	3.13	4.31
16	23000	145000	4.36	5.16
17	14770	19670	4.17	4.29
18	19800	90000	4.30	4.95
19	400	5500	2.60	3.74
20	67740	146500	4.83	5.17
21	56787	142782	4.75	5.15
22	4070	44000	3.61	4.64
24	37789	138740	4.58	5.14
25	531	9700	2.73	3.99
26	7400	8477	3.87	3.93
27	94000	118500	4.97	5.07
28	81489	134252	4.91	5.13
29	12700	14700	4.10	4.17
30	20710	24771	4.32	4.39
31	5000	9800	3.70	3.99
32	18780	116000	4.27	5.91
33	13400	20400	4.13	4.31
34	40000	140110	4.60	5.15
35	4180	5800	3.62	3.76
36	44400	111700	4.65	5.05
37	21500	48444	4.33	4.69
38	36340	49700	4.56	4.70
39	87761	97480	4.94	4.99
40	87487	113782	4.94	5.06

Chart.2 Enumeration of Total bacterial count and total Vibrio count in digestive system of Fresh water prawn *Macrobrachium rosenbergii*. (Set=2; n=60)

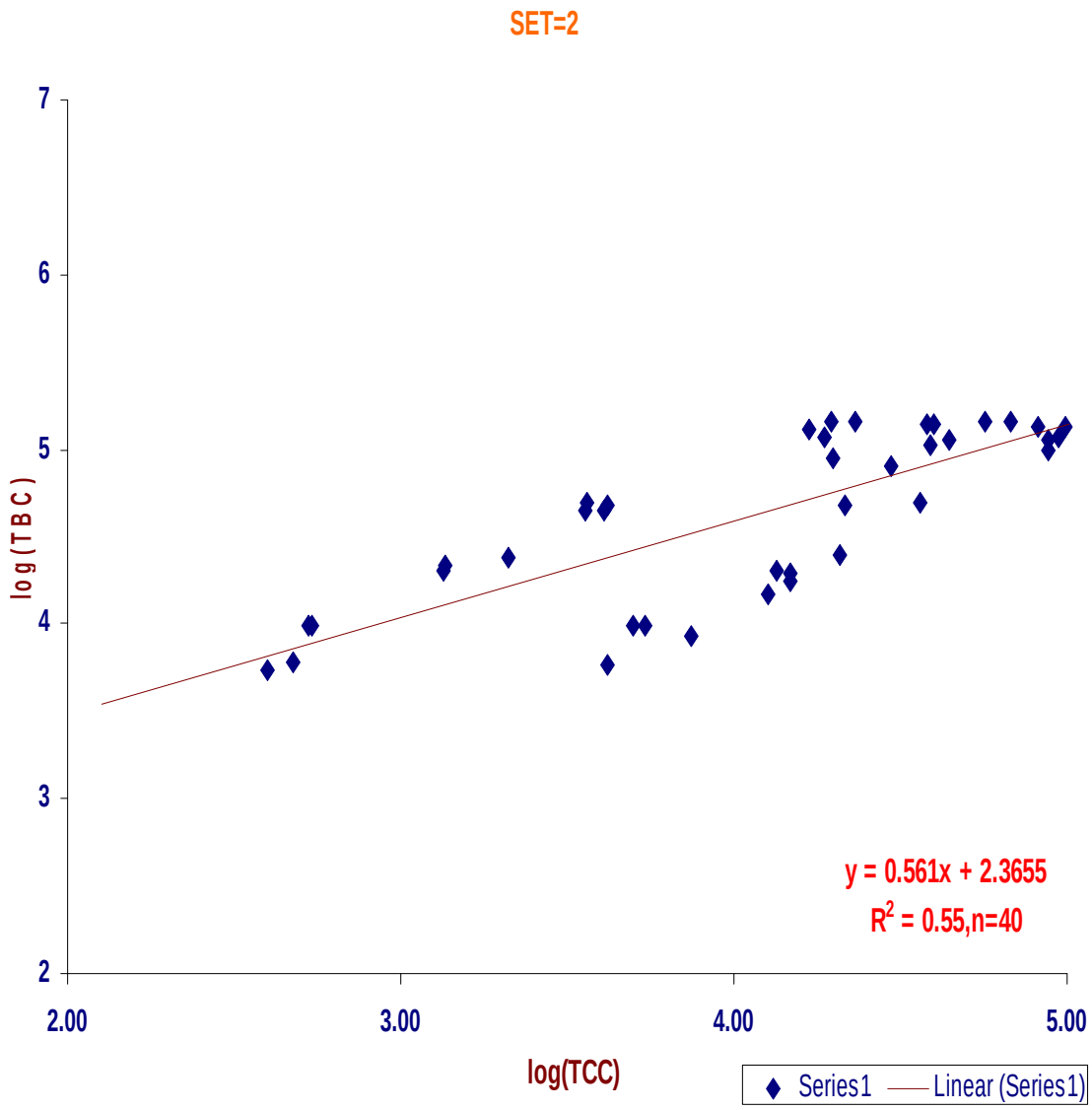

Table.2 Bacteriological characteristics of prawn samples collected from different places (Set=1 and Set=2).

Sl. No.	Characteristics	Set 1 (n=60)	Set 2 (n=40)
1.	TBC range (CFU/ml)	5800 – 145000	5500 – 146500
2.	TCC range (CFU/ml)	430 – 80000	400 – 98700
3.	TBC geometric mean (CFU/ml)	42348.76	41433.68
4.	TCC geometric mean (CFU/ml)	7372.69	11287.78
5.	Relative index p_i		
	TCC/TBC ratio	0.17	0.27
6.	Correlation co-efficient (r)		
	(b) log TCCxlog TBC	0.40	0.55

(TBC = Total bacterial count; TCC = Total coliform count; CFU = Colony forming units)

Table.3 Characterization of *Staphylococcus aureus*

S.No.	Name of the Test	Result
1.	Catalase	Positive
2.	Oxidase	Negative
3.	Coagulase	Positive
4.	Indole	Negative
5.	Methyl red	Positive
6.	Voges Proskauer	Positive
7.	Citrate	Positive
8.	Urease	Positive
9.	Gelatinase	Positive
10.	Nitrate	Positive
11.	Mannitol fermentation	Positive

Table.4 The number and percentage of bacterial isolates from Fresh water prawn (*Macrobrachium rosenbergii*) (set=1;n=60 and set=2;n=40)

S. No.	Bacterial species isolated	No. of isolates Set=1; n=60	Percentage of isolates	No. of isolates Set=2; n=40	Percentage of isolates
1.	*Staphylococcus aureus*	21	35.0	14	35.0
2.	*Pseudomonas aeruginosa*	11	18.3	12	30.0
3.	*Escherichia coli*	29	48.3	16	40.0
4.	*Vibrio sps*	16	26.6	11	27.5
5.	*Enterobacter sps*	9	15.0	10	25.0
6.	*Aeromonas sps*	10	16.6	8	20.0

Chart.3 The number and percentage of bacterial isolates from Fresh water prawn (*Macrobrachium rosenbergii*) (set=1;n=60 and set=2;n=40)

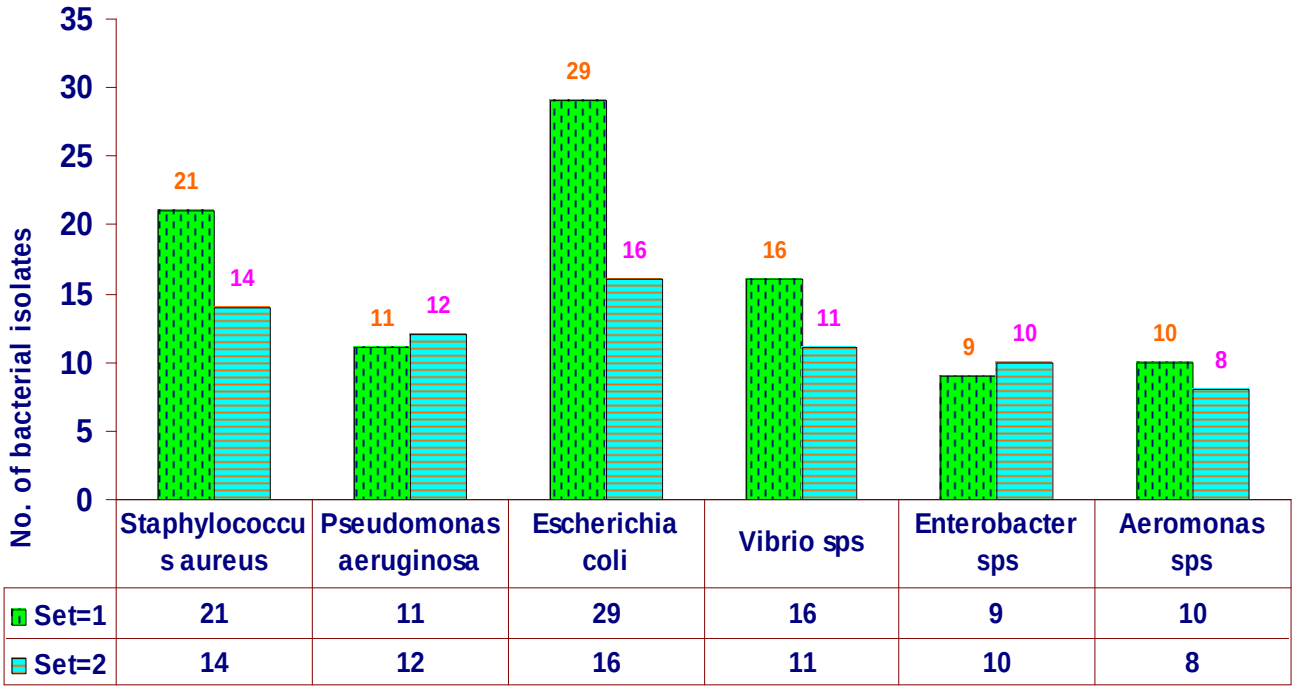

Table.5 Proteolytic activities of isolates in skim milk agar and gelatin liquefaction (set=1;n=60 and set=2;n=40)

S.No.	Bacterial isolates	Proteolytic activity
1	*Staphylococcus aureus*	Positive
2	*Pseudomonas aeruginosa*	Positive
3	*Escherichia coli*	Positive
4	*Vibrio sps*	Positive
5	*Enterobacter sps*	Negative
6	*Aeromonas sps*	Positive

Table.6 Lipolytic activities of isolates in tributyrin agar (set=1;n=60 and set=2;n=40)

S.No.	Bacterial isolates	Lipolytic activity
1	*Staphylococcus aureus*	Negative
2	*Pseudomonas aeruginosa*	Positive
3	*Escherichia coli*	Positive
4	*Vibrio sps*	Positive
5	*Enterobacter sps*	Negative
6	*Aeromonas sps*	Positive

Escherichia coli occupying a less niche in a pond reared system. Geographical variation and mode of sampling where the whole prawn or other parts had been used as the sample could be the reasons for this difference.

Higher bacterial population and the bacterial genus in stomach contents has been observed by Dall (1968) and Moriarty (1976) in prawns which were feeding upon the epiflora and epifauna and the organic matter rich in bacteria from mud substrates.

Moriarty (1976) commented that most of the pelleted feed given as supplementary diet to pond reared prawns was supporting good bacterial growth. As a consequene, when the prawns are put under goes Vibrio may behave as opportunistic pathogens invading tissues and haemolymph through the intestinal wall as suggested by Davis and Sizemore (1982).

The results showed the bacterial genera from 100 prawns samples collected in two different places set=1; n=60 and set=2, n=40. About 45 isolates of Escherichia coli in set=1 and set=2 prawn samples was isolated and characterized. Other genera include Pseudomonas sps (23 isolates), Vibrio sps (27 isolates), Enterobacter sps (19 isolates), Aeromonas sps (18 isolates) and Staphylococcus sps (35 isolates) have been isolated and characterized.

The generic composition of the bacterial flora isolated in the present study varied six genera. Majority of the bacteria isolated were Gram-negative. In prawn samples Escherichia coli, Pseudomonas aeruginosa, Vibrio, Aeromonas sps, Enterobacter sps and Staphylococcus aureus represented the dominant flora. Establishing the microbial profile present in a normal state of the environment will help us recognize the beneficial/harmful potential of autochthonous flora when the environment is stressed or the animals infected. *Escherichia coli*, *Virbio* sps, *Pseudomonas aeruginosa* and *Aeromonas* sps have been reported by Joborn *et al*., 1997; Moriarty,

1998). Austin and Allen (1982), Colorni (1985) and Anderson *et al*. (1989) isolated a similar flora from *M. rosenbergii* hatchery. Sahul Hameed *et al*., (2003) reported *Vibrio* spp. to be the dominant taxon in eggs, larvae and post-larvae of *M.rosenbergii*.

The results of this study present a baseline data of the normal flora that is associated in the hatchery system and marketed area of *M. rosenbergii*. Most bacterial infections are due to opportunistic bacteria that usually are part of the autochthonous flora. The generic composition of the bacteria in the aquatic environment influences the species that colonise the surface or gut of the animal living in the environment.

In the present study bacterial genera were produce protease and lipase enzyme due to hydrolysis of casein and tributyrin on the respective medium in vitro conditions. It was summarized in Table.11 and Table.12. Vibrio are equipped with protease, lipase and therefore are capable to take part in the digestion of entire food. It can be postulated that the Vibrio inhabiting the gut of pond reared *Macrobrachium rosenbergii* play dual roles, both beneficial as well as harmful, in the life of the prawn. Being capable of producing various hydrolytic enzymes they may enhance the digestive process (Bright Sigh, *et al* 1998). However, during adverse environmental conditions they may invade the haemolymph form the intestinal lumen and if the stress factors persist longer, septicemia may result. By-products other than through proteolytic and lipolytic activity would then appear as the contribution of these genera toward spoilage.

This study was also carried out with an antibiotic sensitivity to isolates of prawn samples from two different places and subjected to antibiotic sensitivity test by using Ciprofloxacin, Chloramphenicol, Gentamycin, Streptomycin, Oxytetracycline, Ampicillin, Erythromycin. But, the Oxytetracycline antibiotic showed highly sensitive to all bacterial isolates from the prawn samples.

Bacteria can enter the hatchery system by various routes, most importantly through feed, brood stock and rearing water. The qualitative and quantitative data on the microflora associated with the system would help developing effective strategies for pathogen control (Skjermo and Vadstein, 1999). It is important to understand the microflora associated with hatchery systems because the host–microbe interactions have far reaching implications on larval health, development and outbreaks of disease (Olafsen, 2001).

Some of the bacteria may be opportunistic pathogens causing diseases in stressed prawn populations. Thus it would be important to have data on the levels of bacteria and types of bacteria occurring in hatchery systems and market area at different intervals of operation. The bacterial genera were predominantly Gram-negative and comprised of *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp. and *Bacillus* spp. *Escherichia coli* and *Staphylococcus aureus* were the dominant Gram-positive bacteria. This study documents the bacterial flora associated with Macrobrachium hatchery system and market area during a regular normal run. The higher counts of TCC and TBC in prawn samples in the present study indicate the poor practices that may be at farm level or the poor maintenance of water used in hatchery system and washing of prawns in marketed area. Hence, increasing the management practices for prawn farming to deal with

disease and environmental problems are likely to be the best way.

In conclusion, overall prawn disease was mainly caused by bacteria with various species at different time in year. Fouling bacteria such as proteolytic and lipolytic were mainly detected in prawn digesting systems. Escherichia coli was found to be dominant species isolated, while Staphylococcus aureus, *Aeromonas* sp., Pseudomonas aeruginosa, Escherichia coli, Vibrio sp. and Enterobacter aerogenes, were isolated from prawns. Hence, Coliforms were involved in great number and should be contaminated with water and some other way. So, this should be avoid by proper sanitation and control of bacterial numbers in rearing hatchery and marketed areas. A great number of antibiotics were employed for bacterial treatment, in which Gentamycin and Oxyetracycline are determined to be effective antimicrobial compounds. Knowledge of the qualitative and quantitative aspects of bacterial flora in the hatchery would help to understand disturbances, if any, brought about during disease outbreaks.

References

Abraham, T.J, Manley, R, Palaniappan, R, and Dhevendaran, K.1997. Pathogenicity and antibiotic sensitivity of luminous *Vibrio harÕeyi* isolated from diseased penaeid shrimp. J. Aquat. Trop. 12 :1–8.

Anderson, I.G, Nash, G and Shariff, M., 1990. Mass larval mortalities ingiant fresh water prawn, *Macrobrachium rosenbergii* de Man, cultured in Malaysian modified static 'green water' systems. J. Fish. Dis. 13, 127–134.

Anderson, I.G., Shamsudin, M.N, and Nash, G., 1989. A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrobrachium rosenbergii*, hatcheries in Malaysia. Aquaculture 81, 213–223.

Aquacop. 1977. Observations on diseases of crustacean cultures in polynesia. Proc. World Maric. Soc. 8:685–703.

Austin, B., Allen, D.A., 1982. Microbiology of laboratory hatched brine shrimp (Artemia). Aquaculture.26:369–386.

Baticados, M.C.L., Lavilla-Pitogo, C.R., Cruz-Lacierda, E.R., de la Pena, L.D., Sunaz, N.A., 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Dis. Aquat. Org. 9, 133–139.

Bright Sigh,I.S, P.Lakshmanaperumalsamy and D.Chan dramohan. 1998. Bacterial flora of pond reared Penaeus indicus (Milne Edwards). J.Aqua.Trop.13:133-142.

Colorni, A., 1985. A study on the bacterial flora of giant prawn, *Macrobrachium rosenbergii*, larvae fed with Artemia salina nauplii. Aquaculture. 49: 1–10.

Colorni, A., 1985. A study on the bacterial flora of giant prawn, *Macrobrachium rosenbergii*, larvae fed with Artemia salina nauplii. Aquaculture. 49: 1–10

Dall, W. 1968. food and feeding of some Australian penaeid shrimp. FAO. Fish..Rep.2:251-258.

Davis, J.W and H.K.Sizemore.192. Incidence of Vibrio sps associated with blue crab (Callineetes sapidus) collected from Galveston Bay, Texas. Appl.Environ. Micorbiol. 43:1092-1093.

De la Pena, L.D, Tamaki, T, Momoyama, K, Nakai, T, and K. Muroga. 1993. Characteristics of the causative bacterium of vibriosis in the kuruma prawn, *Penaeus japonicus*. Aquaculture. 115:1–12.

Frappaolo, P.J., Guest, G.B., 1986. Regulatory status of tetracyclines, penicillin and other antimicrobial microbials drugs in animal feeds. Journal of Animal Sciences 62, 86-92.

Frappaolo, P.J., Guest, G.B., 1986. Regulatory status of tetracyclines, penicillin and other antimicrobial microbials drugs in animal feeds. Journal of Animal Sciences 62, 86-92.

Gilmour, A., McCallum, M.F., Allen, M.C., 1975. Antibiotic sensitivity of bacteria isolates from the canned eggs of the California brine shrimp (Artemia salina). Aquaculture 6, 221– 231.

Herwig, R.P, Gray, J.P, and Weston, D.P. 1997. Antibacterial resistant bacteria in surficial sediments near salmon net-cage farms in Puget Sound, Washington. Aquaculture 149, 263–283.

Jiravanichpaisal, P., Miyazaki, T, and G. Limsuwan. 1994. Histopathology, biochemistry and pathogenicity of *Vibrio harĺeyi* infecting black tiger prawn *Penaeus monodon*. J. Aquat. Anim. Health 6, 27–35.

Joborn, A., Olsson, C., Westerdahl, A., Conway, P.Z., Kjellberg, S., 1997. Colonisation in the fish intestinal tract and production of inhibitory substances in internal mucus and faecal extract by Carnobacterium sp. Strain K. J. Fish Dis. 20, 383–392.

Karunasagar, I, Pai, R., and G.R.Malathi,. 1994. Mass mortality of Penaeus monodon larvae due to antibiotic resistant Vibrio harveyi infection. Aquaculture 128, 203– 209.

Karunasagar, I, Pai, R., and G.R.Malathi,. 1994. Mass mortality of Penaeus monodon larvae due to antibiotic resistant Vibrio harveyi infection. Aquaculture 128, 203– 209.

Kerry, J, Hiney, M, Coyne, R, NicGabhainn, S, Gilroy, D, Cazabon, D, and P Smith.1995. Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms. Aquaculture. 131:101–113.

Kerry, J., Nicgabhainn, S., and P.Smith.1997. Changes in oxytetracycline resistance of intestinal microflora following oral administration of this agent to Atlantic Salmon *Salmo salar* L..smolts in a marine environment. Aquaculture 157, 187–195.

McPhearson, R.M., DePaola, A., Zywno, S.R., Motes Jr., M.L., Guarino, A.M., 1991. Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. Aquaculture.99:203–211.

Moriarty, D.J.W. 1976. Quantitative studies on bacteria and algae in food of mullet (mugil cephalus) and the prawn metapenaeus bennetae(Recek and Dall). J.Exp.Mar.Biol.Ecol.22:131-143.

Nygaard, K, Lunestad, B.T, Hektoen, H, Berge, J.A, and Hormazabal, V. 1992. Resistance to oxytetracycline, oxolinic acid and furazolidone in bacteria from marine sediments. Aquaculture.104, 31–36.

Olafsen, J.A., 2001. Interactions between fish larvae and bacteria in marine aquaculture. Aquaculture 200, 223–247.

Phatarpekar, P.V., Kenkre, V.D., Sreepada, R.A., Desai, U.M., Achuthankutty, C.T., 2002. Bacterial flora associated with larval rearing of the giant freshwater prawn, *Macrobrachium rosenbergii*. Aquaculture 203, 279–291.

Prayitno, S.B and J.W.Latchford. 1995. Experimental infections of crustaceans with luminous bacteria related to *Photobacterium* and *Vibrio*. Effect of salinity and pH on infectiosity. Aquaculture.132:105–112.

Roberts,R.J.1978. Fish pathology, Billiere Tindall, London, 318 p.

Sahul Hameed, A.S, K.H. Rahaman, A. Alagan and K. Yoganandhan.2003. Antibiotic resistance in bacteria isolated from hatchery-reared larvae and post-larvae of *Macrobrachium rosenbergii* Aquaculture 217:39– 48

Sahul Hameed, A.S, and G. Balasubramanian. 2000. Antibiotic resistance in bacteria isolated from Artemia nauplii and efficacy of formaldehyde to control bacterial load. Aquaculture 183, 195– 205.

Shewan, J. M. 1971. The microbiology of fish and fishery products-a progress report. J. Appl. Bacteriol. 34:299-315.

Skjermo, J., Vadstein, O., 1999. Techniques for microbial control in the intensive rearing of marine larvae. Aquaculture 177, 333–343.

Spanggaard, B, Jørgensen, F, Gram, L, and H.H, Huss.1993. Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. Aquaculture 115, 195–207.

Tung, C.W., Wang, C.S, and Chen, S.N., 1999. Histological and electron microscopic study on Macrobrachium muscle virus (MMV) infection in the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in Taiwan. J. Fish Dis. 22, 319–323.

Vanderzant, C, Moroz, E and R. Nickelson.1970. Microbial flora of Gulf of mexico and pond shrimp. J.Milk Fd. Technol. 33:346-350.