



## Assessment of the Bacterial Flora in Hemolymph and Hematological Changes in Bacteria-Infected Fresh Water Crab *Paratelphusa Hydrodromas* (Herbst 1974)

## KEYWORDS

haemolymph, fresh water crab, bacterial flora, hematology, plasma

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**ABSTRACT**

The 120 fresh water crab *Paratelphusa hydrodromas* collected from nearby Bhavani River paddy fields, Tamilnadu, India. Healthy and active crab was taken for this study. Randomly 40 crabs were selected, sampled over a 3-months period. Normally the crab hemolymph was sterile (Haskell S., 1975, Stewart et al.). The hemolymph of 75% of all crabs sampled was found to be sterile. Only 25% of the crab hemolymph sample was found bacteria. Despite only bacteria containing individuals had very low amount of plasma protein ( $29.52 \pm 0.50$ ) and Carbohydrate ( $6.65 \pm 0.37$ ) in hemolymph. Three bacterial floras named as *Pseudomonas*, *Bacillus* and *Staphylococcus* were isolated from hemolymph and identified by using various biochemical tests. The present study supported by many researchers, but most of the studies were carried out only in marine crab and shrimp.

**INTRODUCTION**

It is generally the circulatory system of healthy animals is sterile, whereas the Presence of microorganisms is usually considered to be a sign of disease (Haskell s., 1975). In normal conditions; crustaceans maintain a healthy state and keep infections under control. The presence of antimicrobial compounds in the haemolymph of crustacean species (crabs) against most of the microbial infections has been reported by so many researchers (L. Khoo et al., 1999. A. Veeruraj et al., 2008. J. R. S. Chisholm et al., 1992.). Externally, crab were covered by a hard, rigid exoskeleton that functions as a well-organized physico-chemical barrier against mechanical injury and microbe invasion. Their gastrointestinal tract is another important route for pathogen invasion. It is also protected almost entirely by chitinous membranes. This cuticular coat, in combination with an acid environment rich in digestive enzymes, is able to inactivate and degrade most viruses and bacteria (Jiravanichpaisal et al., 2006). However, once the cuticle barriers are disrupted, pathogenic and other opportunist microorganisms can enter into the hemocoel and thus turn on the internal immune defenses of the crustacean. In aquatic environment contain verities of organism also rich in microorganisms; crustaceans have developed effective system for detecting and eliminating noxious microorganisms. The aquatic animals are in intimate contact with aquatic environment rich in pathogenic bacteria and are prone to infection by those microbes at various stages of growth, and losses due to infection (D. A. Hudson and R. J. G. Lester 1994). In the present study, attempted to assess the hematological changes occurring in bacteria-infected crab with respect to changes in total protein and glucose content. Also using biochemical tests to find out what are the bacterial floras found in haemolymph of fresh water crab *P. hydrodromas*.

**MATERIALS AND METHODS****Sample collection**

The Healthy crabs collected nearby Bhavani river paddy fields, Bhavani, Tamilnadu, India. They were transported and

kept for acclimatization in rectangular tank, for a period of one week. Before stocking, the tank was washed with clean water several times. Finally, the tank was washed with 0.1%  $\text{KMnO}_4$  for disinfection. Before introducing into the tank, the crabs were screened for any visible pathological symptoms. The crabs were bled aseptically by cardiac puncture through the intersegmental membrane between the posterior of the carapace and the abdomen. To avoid hemocyte degranulation and coagulation, the hemolymph was collected in sodium citrate buffer, pH 4.6 (2:1, V/V) to which equal volume of physiological saline (0.85%, NaCl, w/v) was added to the one portion of collected hemolymph for biochemical study and another portion of hemolymph was used for the microbial isolation.

Agar plates were streaked from tubes of the highest dilution showing growth. Isolates were selected from the streak plates by picking the colony representing the predominant colony type on the plate. Usually the highest dilution appeared to be a pure culture of a single organism. The isolated bacteria were subcultured and biochemically identified according to the Bergey's manual (Bergey'et al, 1984).

**Biochemical analysis**

The protein content of crude and precipitated and purified haemolymph samples were estimated total protein (TP) and the total carbohydrate (TC) concentrations in haemolymph were determined according to the methods of Lowry et al. 1951. and Roe 1955.

**RESULT:**

From the freshwater crab, the hemolymph samples were collected aseptically for biochemical estimations (protein and Carbohydrate) were done in which most of the hemolymph samples exhibit the uniform concentration of protein ( $68.96 \pm 0.37$ ) and glucose ( $15.45 \pm 0.20$ ) in all of the forty specimens. Whereas, few infected crabs showed decreased concentration of protein ( $29.52 \pm 0.50$  and glucose ( $6.65 \pm 0.37$ ) level. The decreased amount of protein and Car-

bohydrate were found the infected crab individuals only. The biochemical contents acts as an indicator for infections as far as the concentration amount is concerned.

The freshwater crab does not exhibit any sign of infection at external morphology. Only at the very serious state, the infection can be identified and observed externally. This is due to the anatomy and physiological nature of the crab. As it is being covered by hard carapace the internal health condition of the crab is not manifested over it.

For microbiological investigations, the hemolymph samples were plated on to Mc Conkey agar and nutrient agar medium. Most of the bacterial population (75%) exhibited no growth (sterile). 25% of the crab showed microbial population in their hemolymph.

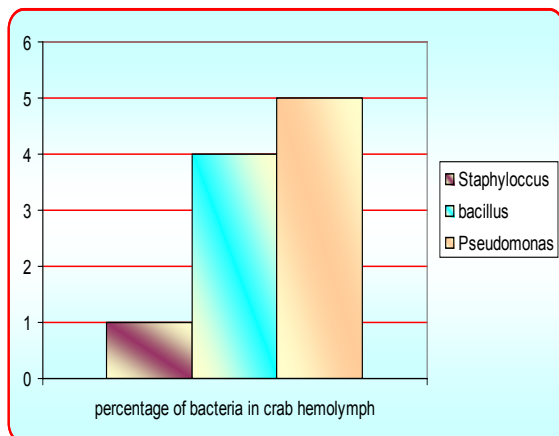
The bacterial isolates were identified, based on the standard biochemical characterization which is exhibited in **Table-A**.

**Table – A: Biochemical Test for Bacterial Identification**

Tests	Bacterial Culture			
	1	2	3	
<b>Gram Staining</b>	+Cocci	+Rod	-Rod	
<b>Indole test</b>	-	-	-	
<b>Methyl red test</b>	+	-	-	
<b>Voges – Proskauer test</b>	+	+	-	
<b>Citrate utilization test</b>	-	-	+	
<b>H<sub>2</sub>S production test</b>	-	-	-	
<b>Urea utilization test</b>	-	-	-	
<b>Catalase test</b>	+	+	+	
<b>Nitrate reduction test</b>	+	+	+	
<b>Oxidase test</b>	-	-	+	
<b>Starch hydrolysis test</b>	-	+	-	
<b>Casein hydrolysis test</b>	+	+	-	
<b>Gelatin hydrolysis test</b>	+	+	+	
<b>Triple – sugar iron agar test</b>				
<b>Acid production</b>	<b>Butt</b>	-	-	-
	<b>Slant</b>	+	-	+
<b>Alkaline condition</b>	<b>Butt</b>	+	+	+
	<b>Slant</b>	-	+	-
<b>Gas production</b>	G	G	G	
<b>H<sub>2</sub>S production</b>	-	-	-	
<b>Carbohydrate fermentation</b>				
<b>Glucose</b>	+	+	-	
<b>Lactose</b>	+	-	-	
<b>Mannitol</b>	+	+	-	
<b>Sucrose</b>	+	+	-	

The existence of microbes in the freshwater crab *Paratelphusa hydrodromous* is presented in **Figure 1**.

**Figure – 1: Percentage of the existence of microbes in the freshwater crab *Paratelphusa hydrodromous***



## DISCUSSION

It is generally the circulatory system of healthy animals is sterile, whereas the Presence of bacteria is usually considered to be a sign of disease (haskell s., 1975). The examination of the 2,035 lobsters, hemolymph was Sterile in about 75 %;( Stewart et al. 1972). Crabs are the great resource of antimicrobial proteins with extensive range of antimicrobial properties which is highly supported in the haemolymph study of *C. lucifera*. (Rameshkumar et al. 2009., S. Ravichandran, K. Sivasubramanian and R.M. Anbuhezian 2010)). In invertebrates, the circulating hemocyte has a major role in the protection of the animal against aggressive microorganisms by participating in recognition, melanization, phagocytosis, and cytotoxic activities (Jiravanichpaisal et al., 2006). The haemolymph of crustacean have potent antimicrobial peptide showed diverse array against several human pathogens. Haemolymph of freshwater crab, *Paratelphusa hydrodromous*, exerts very strong activity against the tested microbes *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Arulprakash A., et al.2011). Present work states commonly hemolymph doesn't contain any bacterial flora. Only initial or vigorous conditions of infection period's only microbial flora occurs in the hemolymph. The result is agreed with the above reports.

In the present study, some of the fresh water crab *Paratelphusa hydrodromous* hemolymph samples exhibit decreased level of protein and carbohydrate level. The protein ratio in plasma is an indicator of health status (Chanutin et al. 1938). The decrease in haemolymph protein content might be due to an energy crisis and other stress factors arising from microbial infection (S. Mathew 2009). The *Bacillus* induced a significant ( $P > 0.010$ ,  $t = 13.5873$ ,  $df = 4$ ,  $n = 3$ ) decrease in the protein content of haemolymph, and similar result was observed in reducing sugars in crustaceans hemolymph (Anandakumar, M. D, Ann Sandhya Michael 2011).

Stewart and Cornick (1972) found a significantly decreased amount of glucose from the hemolymph and a reduction in total carbohydrates in *H. americanus* infected with *G. homari*. The decreased amount of glucose which observed in *C. sapidus* may be attributed, at least in part, to uptake and utilization by *P. perniciosus*, resulting from the hosts' inability to compete successfully for their own nutrients, as in the case in lobsters infected with *G. homari*. Floreto et al. (2000) reported a considerably decreased in total protein values in haemolymph of crab for the period of starvation, stress and infection.

Based on the growth and biochemical characterization of the bacterial isolates, we have identified *Pseudomonas* spp, *Staphylococcus* sp and *Bacillus* spp., were found in the hemolymph. This was depicted in Figure-1. Our findings were supported by 25% of other researchers. Bacteria were isolated from the haemolymph of *P. monodon*. More than 50% of these bacteria were *Vibrio* spp., the remainders were *Aeromonas* spp. and *Pseudomonas* spp. Bacteria have also been isolated from the haemolymph of other species of apparently healthy crustacean and other aquatic organisms such as *H. americanu*. Cornick and Stewart, 1966., *Callinectes sapidus* Haskell et al., 1975., Thune and *P. clarkii* Scott 1986 and *M. rosenbergii* Brady and Lasso-de la Vega, 1992. The present study indicated the freshwater crab *Paratelphusa hydrodromous* doesn't contain any pathogens. If have any bacterial flora only at initial stage of infection or vigorous condition, based on the infection, the biochemical (protein and carbohydrates) composition were altered depends upon the microbial population. Further studies to find out the bacteria and host specification and immunological studies like antibody and antigen interaction and other immunological studies.

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