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Isolation and identification of bacteria from local market fresh water crab, *Paratelphusa jacquemontii,* from Amravati district, Maharashtra, India.

Ghaware AU[1] and Jadhao RG[2]*

[1]Assistant Professor, Department of Zoology, Mahatma J. Fule Mahavidyala, Bhatkuli, Dist. Amravati, MS, India
[2]Professor and Head, Department of Zoology, Shri. Shivaji Science College, Amravati, MS, India.
*Corresponding Author, rajusingjadhao@gmail.com

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ABSTRACT

The present study was conducted to evaluate the hygienic quality and freshness of crab *Paratelphusa jacquemontii* (Rathbun) through the investigation of the occurrence of bacteria which is an indicator for crab quality. Crabs were collected every week from local crab market. Carapace and gills of the crab was examined. *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis, Klebsiella pneumonia*, *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Staphylococcus aureus* were identified by Biochemical tests (IMViC Tests). Among the seven bacterial species *E. coli. Vibrio cholera* and *K. pneumonia* were found in all the collected samples. The result of this study revealed that raw crab sold in local crab market has high contamination so the presence of the bacterial species has strongly suggested that there is need for innovative measures to discourage the local population from eating improperly cooked crabs.

Keywords: *P. jacquemontii*, Bacterial isolation, Fresh water crab.

INTRODUCTION

Fresh water crab provides high quality protein and it also contains omega-3 fatty acids that afford potential health benefits. Crab meat contains several essential vitamins and minerals, such as vitamin B12, which is necessary for proper nerve function. These crustaceans are rich in the minerals, zinc and copper, which are important for various vital bodily functions. Consumption of infected crab may cause diseases due to infection or intoxication. Some of these diseases are caused by the microorganisms present on the external surfaces including carapace, gills and the gut of the crab. In water microorganisms are kept away from invading by the normal defense mechanism of crab, when they are alive. Outside the water or on death, the microorganisms or the enzymes they secrete are free to invade or diffuse into the body parts where they react with complex mixture of natural substan-

-ces present resulting in a well- defined sequence of changes in odoriferous and flavourous compounds. The bacteria present on the crab are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions (ICMSF 2007). The outlined and discussed the hazards and challenges associated with handling crab during farming, capture and the environmental contaminants in seafood that may pose a risk to human health (Yagoub 2009). The quality of the raw crab (fresh or iced) at markets varies so widely that there is an obvious need for developing quality standards. There is no study on Microbial identification of these crabs. Therefore this study was carried out to identify certain microbial analysis to assess the quality and freshness of raw crab (*P.jacquemontii*) sold at a market in Amravati, Maharashtra, India.

MATERIALS AND METHODS:

Laboratory analysis

Crab samples

Crabs (P.jacquemontii) were collected from a local crab market in Amravati. Totally 10 numbers of samples were collected every week from November 2019 to February 2020. The collected samples were aseptically and immediately transported in a thermal bag to the laboratory and processed within 3 h of acquisition, and samples were kept in the refrigerator (4–8 °C).

Sample preparation

The 10 g of the crab body parts sample was cut with a sterile knife. The cut body parts were crushed into small pieces in a sterile mortar with about 10 ml sterile water. From the crushed sample, 1 ml aliquot volume was measured out and homogenized in a clean, dry sterile beaker containing 9 ml of distilled water giving a 1:10 dilution. This was done for the 10 crab samples.

Isolation and identification bacteria form crab body sample of *P.jacquemontii*

The collected samples were processed in the laboratory according to the standard microbiological methods under complete aseptic conditions. The swabs were inoculated on nutrient agar and incubated at 37 °C under aerobic condition. The isolated bacterial colonies were identified on the basis of their morphological, physiological and biochemical characters. These culture were subjected to various biochemical tests such as gram staining, motility, indole, methyl red, voges proskauer, citrate, Triple sugar ion, oxidize, carbohydrate fermentation, hydrogen sulfide production tests for identification of phosphate solubilizing bacteria using Bergey's manual of systematic bacteriology (Holt et al.1994). The bacteria such as *E. coli, K. pneumonia, P. vulgaris, B. subtilis, P. aeruginosa, Vibrio parahaemolyticus* and *S. aureus* were isolated from the crab samples.

RESULTS AND DISCUSSION

In bacteria, bio-oxidation reactions are very important. These reactions help bacteria to provide energy by oxidation of organic substances or by fermentation. Based on these reactions, the bacteria were identified and the results were presented in (Tables: 1-4).

Table 1: Bacteria isolated from fresh water crab *P. jacquemontii* purchased on first week of November 2019.

Testing	Microorganisms			
	Escherichia coli	*Vibrio parahaemolyticus*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*
Gram staining	−	-	−	+
Motility	+	+	−	−
Indole production	+	+	−	+
Methyl red	+	-	−	−
Voges-Proskauer	−	-	+	−
Citrates	−	+	+	+
Triple sugar iron	−	+	−	−
Oxidase	−	+	−	D
Carbohydrate fermentation	+	+	D	D

+: Positive, −: negative, D: different

Table-2: Bacteria isolated from fresh water crab *P.jacquemontii* purchased on first week of December 2019.

Testing	**Microorganisms**				
	Escherichia coli	*Vibrio parahaemolyticus*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Bacillus subtilis*
Gram staining	–	-	–	–	+
Motility	+	+	–	+	–
Indole production	+	+	–	+	+
Methyl red	+	-	–	+	–
Voges-Proskauer	–	-	+	–	–
Citrates	–	+	+	+	+
Triple sugar iron	–	+	–	+	–
Oxidase	–	+	–	D	D
Carbohydrate fermentation	+	+	D	D	D
Catalase	+	+	+	+	+

+: Positive, –: negative, D: different

Table-3: Bacteria isolated from fresh water crab *P.jacquemontii* purchased on first week of January 2020.

Testing	**Microorganisms**			
	Escherichia coli	*Vibrio parahaemolyticus*	*Klebsiella pneumonia*	*Proteus vulgaris*
Gram staining	–	-	–	–
Motility	+	+	–	+
Indole production	+	+	–	+
Methyl red	+	-	–	+
Voges-Proskauer	–	-	+	–
Citrates	–	+	+	+
Triple sugar iron	–	+	–	+
Oxidase	–	+	–	D
Carbohydrate fermentation	+	+	D	D
Catalase	+	+	+	+

+: Positive, –: negative, D: different

Table-4: Bacteria isolated from fresh water crab *P.jacquemontii* purchased on first week of February 2020.

Testing	**Microorganisms**				
	Escherichia coli	*Vibrio parahaemolyticus*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Gram staining	–	-	–	+	+
Motility	+	+	–	–	–
Indole production	+	+	–	+	–
Methyl red	+	-	–	–	–
Voges-Proskauer	–	-	+	–	–
Citrates	–	+	+	+	+
Triple sugar iron	–	+	–	–	–
Oxidase	–	+	–	–	–
Carbohydrate fermentation	+	+	D	D	D
Catalase	+	+	+	+	+

+: Positive, –: negative, D: different

In this study, *E. coli, Vibrio parahaemolyticus ,K. pneumonia, P. vulgaris, B. subtilis, P. aeruginosa* and *S. aureus* were identified from the crab. *E. coli, Vibrio parahaemolyticus* and *K. pneumonia* were found in all the monthly samples (Tables: 1, 2, 3, and 4) Whereas, *B. subtilis* (Tables: 2) and *P. aeruginosa* (Tables: 1 and 3) were identified two times. *P. vulgaris* (Tables: 2 and 3) and *S. aureus* (Tables: 4) was identified. The crabs are highly consumable and prone to vast variations in quality due to differences in species, environmental habitats, and feeding habits. They can also function as carriers of several microbial and other health hazards. Therefore maintenance of quality is most important in production and trade of crab products. Although only a few infectious agents in crab are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health due to the consumption of raw or insufficiently processed crabs and crab products. In this study, *E.coli., Vibrio Parahemolyticus,* and *Klebsiella pneumonia* sp. were isolated from all of collected crab samples is of highly importance because this bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food quality as spoilage organism. The consumption of these disease or infected crabs possesses greater health risks than the consumption of apparently healthy ones. The general public and consumers of crabs should therefore ensure that they do not buy or consume disease or injured crabs. Organic materials of any type are suitable foodstuffs for bacteria growth. A case of cholera occurred in a patient in Maryland, who had eaten crab harvested commercially along the Texas coast in October 1984. Findings of *Vibrio sp.* in the tissue of crabs of present studies are considered to be correlated with the epidemiology and transmission of cholera in the aquatic environment.

This is in accord with previously mentioned by (Jeyasekaran *et al*. 2006, and Koutsoumanis and Nychas 2000). In this study seven bacterial species were isolated form *P.jacquemontii.* Among the seven bacterial species *E. coli*. *V. parahaemolyticus* and K. *pneumonia* were found in all the samples and others were not found. Apart from the enteric- organisms, *S. aureus* encountered in this study are known enterotoxin producing agent and a microorganism which is poisonous. This is in agreement with the previous study by some authors in Nigeria and outside Nigeria (Okonko et. al.2008, 2009). The results from this study and according to published microbiological guidelines as cited by (Gilbert et al.1996) suggest that the microbiological quality of the crab examined is unacceptable and pose a potential risk to public health. The diversity of potential pathogens from the samples of crab is of concern particularly at a time when many in our communities are immunologically compromised as a result of various illnesses (Mhango et al.2010). From this investigation, it can be concluded that these characteristics of water bodies are influenced by seasonal variations. It is recommended that the proper maintenance of the captured crabs is necessary. Proper sanitation measures and environmental education to public care essential to keep these crab bodies clean and safe. There is need for innovative measures to discourage the local population from eating improperly cooked crabs.

Conflicts of Interest: The authors declare no conflict of interest.

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