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CASE REPORT

TO HIGHLIGHT THE QUALITY OF EDIBLE OYSTER, (*CRASSOSTREA GRYPHOIDES*) WITH RELATION TO MICROFLORA OF KELVA MARKET, PALGHAR, MAHARASHTRA

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ABSTRACT

This study has been undertaken to investigate the microflora of edible oyster (*Crassosstrea gryphoides*) from Kelva market, Palghar. In the present study between 2016 to 2018, total 12 samples were collected and processed for different microbial flora. In all 64 isolates were isolated. Maximum number were observed of *Aeromonas spp.*(42%) followed by *Pseudomonas spp.*(28%), *Enterobacter kobei* (14%), *Providencia vermicola* (13%) and *Shewanella algae* (03%).In this paper an attempt is being made to enumerate the different microbes and their consequences on the human health if any.

INTRODUCTION

It was not until the late 19th to early 20th century that public health agencies considered controls to reduce shellfish-borne disease (US Dep. Health Hum. Servo 1993). In February 1925, the Surgeon General arranged a conference with the Bureau of Chemistry (now the United States Food and Drug Administration) and the Bureau of Commercial Fisheries (now the National Marine Fisheries Service) to establish sanitary controls for the oyster industry. At this conference the agencies resolved to control "the beds on which shellfish are grown" and "the plants in which shellfish are shucked" (US Dep. Health Hum. Servo 1993). Shellfish safety issues continue to revolve around these two categories: the quality of the waters in which shellfish are grown, and the conditions under which shellfish are harvested, processed, and distributed (R. J. Wittman and G. J. Flick., 1995). Significant strides have been made in creating a safer food, but many problems nevertheless remain (R. J. Wittman and G. J. Flick., 1995). We examine first the magnitude of the shellfish-borne disease in terms of its prevalence and the risks associated with shellfish consumption (R. J. Wittman and G. J. Flick., 1995). We next address the issues of water quality, harvesting, processing, and distribution as they relate to shellfish-borne disease, and present strategies to minimize the risk of disease (R. J. Wittman and G. J. Flick.,1995). Sanitary controls have focused upon edible bivalve mollusks, including oysters, clams, mussels, and scallops, of the class Pelecypod a since they are filter feeders and concentrate pathogens from the water (Metcalf TG. 1975). During natural climatic problems, fishing catch get reduced or stop completely. This increased the demand of edible oyster because of easily available and affordable cost for the people in general and poor in particular. Present study is an attempt to highlight the quality of edible oysters with relation to microflora.

Study area

Kelva fish market was choosen for this study. Kelva is the tourist place of Palghar Taluka which is situated at the costal area of Arabian sea having geographic coordinates of 19°37'9"N 72°43'23"E. The market is near the fishermen colony, Figure No. 1 and 2.

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Figure 1 and 2 - Kelva fish market of Palghar Taluka.

MATERIALS AND METHODS

A total of 12 samples of edible oysters (*Crassosterea gryphoids*) were collected from Kelva market during the period from 2016-17. Samples were collected from Kelva market in polythene zip wrap bag, labelledand kept in the ice box and immediately transported from market to the Zoology department research Laboratory of S.D.S.M. college, Palghar within an hour. Further sample were proceed for the Total Plate count and differential pathogens with standard protocol. The 10 gram of edible oyster already depurated from shell was transferred to a sterile beaker to which 90 ml of normal saline solution (NSS) was added. The sample were serially diluted by 10 fold serial dilution method in the normal saline solution up to 10^{-7}. The 10^{-7} dilutions were used in 0.1 ml quantities for the Standard Plate count (SPC) on Plate count agar (PCA). The agar plates were inoculated by pour plate method and incubated at 37^0C for 24 hrs. The 10^{-4} dilutions were taken for plateing following differential media simultaneously during processing of the samples. Baird Parker agar, Slanetz and Bartley agar, Macconkey agar, Violet red bile agar, TCBS agar. Salmonella Shigella agar and Xylos lysine deoxycholateagar were streaked after enriching the sample in selenite cystine broth at 37^0C for 18 hrs. Suspected pathogens were further identified by growth pattern and morphology of the colony, direct microscopic examination and different biochemical tests (corwn and steel, 1993 and Hi-media 1992, 2016, 2018). Authentification of representative organism was carried out at geneOmbio lab Baner, Pune.

RESULT

Total 12 samples of edible oyster (*Crassostrea gryphoides)* were collected from Kelva market and the same were processed for Total viable count (T.V.C) and different pathogenic bacteria. The T.V.C. ranged from 00×10^7 to 60×10^7 (cfu/ml). Out of 12 samples processed, the total of 64 isolates were isolated. *Pseudomonas spp.* and *Aeromonas spp.* Occurred in maximum number of samples (12) followed by *Enterobacter spp.* (09), *Providencia spp.* (08), *Shewanella spp.* (02).The sample wise number is shown in Table-1and % wise pathogenic microbes in Table-2 and figure 3.

Gram Negative Rods

In the present study total 64 isolates were isolated from the 12 samples of edible oysters of the Kelva market (Table-2 and figure-3). *Aeromonas spp.* encounted in maximum samples and constituted more than (42%) followed by *Pseudomonas spp.* (28%), *Enterobacter kobei* (14%), *Providencia vermicola* (13%) and *Shewanella algae* (3%).

Table 1 Pathogenic microbes of edible oyster (*Crassostereagryphoids*) from Kelva Market of Palghar

Sample No.	Name of microbes	Total No. of microbes	T.V.C=×10^7 (cfu/ml)
1	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Providencia vermicola, Aeromonas veronii, Aeromonas taiwanesis* (4), *Enterobacterea kobei*	09	18
2	*Pseudomonas stutzeri, Providencia vermicola, Aeromonas taiwanesis* (2)	04	00
3	*Pseudomonas stutzeri, Providencia vermicola, Shewanella algae, Aeromonasveronii, Aeromonas taiwanesis, Enterobacter kobei*	06	19
4	*Pseudomonas stutzeri, Shewanella algae, Aeromonas taiwanesis* (2), *Eenterobacterea kobei*	05	35
5	*Pseudomonas stutzeri, Providencia vermicola, Eenterobacterea kobei*	03	15
6	*Pseudomonas stutzeri, Providencia vermicola, Aeromonas veronii, Eenterobacterea kobei*	04	15
7	*Pseudomonas stutzeri, Aeromonas taiwanesis*(2), *Eenterobacterea kobei*	04	08
8	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Aeromonas veronii, Aeromonas taiwanesis, Eenterobacterea kobei*	05	60
9	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Providencia vermicola, Aeromonas taiwanesis*(2), *Eenterobacterea kobei*	06	20
10	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Providencia vermicola, Aeromonasveronii, Aeromonas taiwanesis*(2), *Enterobacterkobei*	07	08
11	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Providencia vermicola, Aeromonas veronii, Aeromonas taiwanesis*	05	09
12	*Pseudomonas stutzeri, Pseudomonas sihuiensis, Shewanella algae, Aeromonas taiwanesis* (2), *Aeromonas veronii*	06	30
	TOTAL	64	

Table 2 percentage of different pathogenic microbes isolated from edible oyster (*Crassostreagryphoides*) of Kelva Market, Palghar

Sr. No.	Name of Isolates	Isolates from no. of samples	%
1	*Pseudomonas stutzeri*	12	19
2	*Pseudomonas sihuiensis*	06	9
3	*Providencia vermicola*	08	13
4	*Shewanella algae*	02	3
5	*Aeromonas taiwanesis*	20	31
6	*Aeromonas veronii*	07	11
7	*Enterobacter kobei*	09	14
	G.T.	**64**	**100**

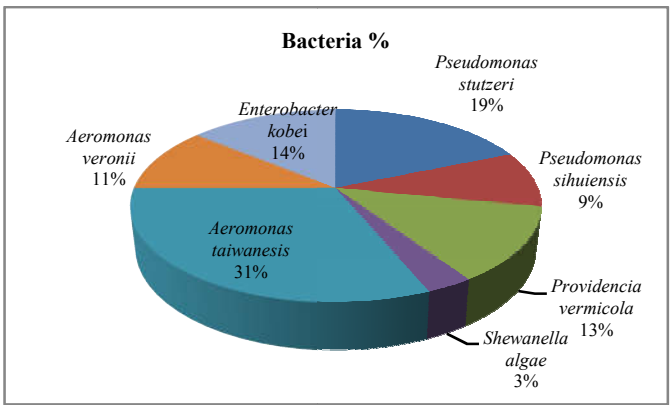

Figure 3 Diagrammatic percentage of different pathogenic microbes isolated from edible oyster(*Crassostrea gryphoides*) of Kelva market, Palghar

DISCUSSION

Gram Negative rods

In the present study only gram negative rods could be isolated from the 12 samples of edible oysters. The maximum number

shared by *Aeromonas spp.* (42%) The genus *Aeromonas* consists of gram-negative rods widely distributed in freshwater, estuarine, and marine environments [Holmes *et al.,* 1996 and Martin-Carnahan *et al.,* 2005]. *Aeromonas* species cause a wide spectrum of disease syndromes among warm- and cold-blooded animals, including fish, reptiles, amphibians, mammals, and humans (Gosling *et al*., 1996 and Janda *et al*., 1996). *Aeromonas* strains are primarily inhabitants of aquatic environments, often associated with fish and human diseases (Figueras, 2005; Martin-Carnahan and Joseph, 2005).The most common clinical presentation of *Aeromonas* is diarrhoea, followed by localized soft-tissue infections and bacteraemia, the prevailing associated species being *A. veronii*, *A. caviae* and *A. hydrophila* (Figueras, 2005). *Aeromonastaiwanensis* is a Gram-negative, oxidase- and catalase-positive, non-spore-forming, motile bacterium of the genus *Aeromonas*isolated from wounds of patients in Taiwan.*(Alperi et al.,2009).*

18 isolates of Pseudomonas spp. were recorded from 12 samples of edible oyster.Out of 18 isolates 12 isolates were of Pseudomonas stutzeri and 06 of Pseudomonaas sihuiensis.P. stutzeri, which belongs to the genus *Pseudomonas*, is widely found in soil, fresh water, oceans and animals. It is an aerobic Gram-negative bacterium and a type of denitrifying bacterium (Lalucat*et al*.,2006). A variety of strains of *P. stutzeri* have been isolated to study environmental bioremediation. *P. stutzeri* is capable of degrading a number of organic pollutants, such as naphthalene (Bosch *et al.,* 1999). *Pseudomonas stutzeriis an aerobic, nonfermenting, active, gram-negative oxidase-positive bacteria. Cases of P. stutzeri infection concern typically immunocompromised patients with underlying diseases or previous surgery (*Noble andOverman 1994*).Pseudomonas sihuiensis* is a novel spp. Isolated from a forest soil in sihui city, South China (Wu, Min *et al*., 2014). No further clinical report is reported of *Pseudomonas sihuiensis* and clear function of this species.

09 isolates of Enterobacter kobei were recorded in the present finding which is 14% of the total isolates. Enterobacter kobei is the species of the *Enterobacter cloacae* complex, which is phenotypically most closely related to the species *E. cloacae (*HaraldHoffmann *et al.,* 2005).

08 isolates of *Providencia vermicola* recorded which is 13% of the total isolates. The enterobacterial genus *Providencia* comprises five species that have been isolated from the colon and faeces of humans (*Providencia alcalifaciens, Providencia rustigianii*), wounds, urinary tract and respiratory tract of humans (*Providencia stuartii*), urinary tract of humans, poultry, faeces from reptiles and other environments (*Providencia rettgeri*) and from faeces of penguins (*Providencia heimbachae*) summarized by (Penner 1991).

Shewanella algae recorded two times and constituted 3% of the total isolates isolated. *Shewanella algae* has been identified as a new bacterial species from clinical samples (Nozue1992). It is a rare human pathogen and symptoms of infection are often misidentified as Vibrio spp. (Myung2009). It can be isolated from a wide range of environments, including fresh water, estuary, and the deep sea (Fu *et al.,* 2014).

The suitable processing and preservation methods are required to prevent the pathogenic microbes particularly in post-harvesting period of oyster (Seaman, 1991 and Aaraas*et al*., 2004). In many countries, cold storage temperature is generally considered as useful preservation methods before sell and consumption (Seaman, 1991 and Aaraas *et al*., 2004). As example, Australian Shellfish Quality Assurance Programme (ASQAP) recommended that oyster must be stored at ≤10°C for 24 hrs before consumption (Fernandez-piquer *et al*., 2012). However, consistence refrigeration is difficult to achieve along the entire oyster supply chain, particularly difficult in the developing countries (Madigan, 2008).

CONCLUSION

Edible oyster (*Crassostrea gryphoides*) naturally possessed microflora due to filter feeding. During the odd season, when there is no fish catch, the edible oyster used to be in high demand. But the oyster depuration is being done under unhygienic conditions by traditional methods which is the cause of concern not only from the load and variety point of view microbes but also the health hazards of sellers and consumers.

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