STUDY OF PATHOGENIC MICROBES FROM EDIBLE OYSTER (CRASSOSTREAGRYPHOIDES) OF KELVA COAST;PALGHAR, MAHARASHTRA

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Abstract
Present study on edible oysters (Crassostreagryhoides) was conducted at kelva coast, Palghar, Maharashtra. 12 samples were processed from 2016 to 2018 for bacterial study. A total of 44 isolates were isolated. The occurance of Aeromonas spp. was (29%) followed by Pseudomonas spp. (25%), Enterobacterkobei (23%), Providenciavermicola (14%) and Bacillus flexus (09%). In the present study an attempt was made to enumerate the bacteria along with health hazard if any.

Key words: Pathogenic microbes, Kelva, Health hazards.

Introduction

The edible oysters are very popular as raw and processed food in the South Indian states, particularly in Goa, in the South East Asian countries, Europe, Australia andUSA. Naturally, oyster accumulates microorganisms during the process of filter feeding. These shellfish are prone to harsh environmental contamination by fecal pathogens like Salmonella sp. Shigella sp. and Escherichia coli (Musa et al., 2008). Illnesses due to food-borne contamination frequently occur, but rarely documented. E. coli and Salmonella sp. could extensively spread inside the body of human beings consuming oyster and lead to serious infection to the human and death (Forsythe, 2002). On the other hand, Vibrio sp. may be transmitted to humans by the ingestion of raw seafood. Oysters from these waters are often incriminated in human diseases since the oysters are commonly consumed as raw (Haldy, 1997).

Farely good amount of work has been carried out on edible oyster from different developed countries and rarely from India and not from Palghar district of Maharashtra. Since edible oyster are cheaply available sea food, it is becoming the good source of food to the people in the vicinity specially to the poor. It is intended in this paper to enumerate the pathogenic microbes from the edible oysterCrassostreagryphoides and in turn the health hazards if any.

Study Area

This study was carried out at KelvaCoast, Palghar.Kelva is the tourist place of PalgharTaluka which is situated at the coastal area of Arabian sea having geographic coordinates of 19°37'9"N 72°43'23"E. Figure No. 1 and 2.

Figure.1 and 2:- Kelva coast of PalgharTaluka.

Materials and methods

A total of 12 samples of edible oysters (Crassostereagryphoids) were collected from kelvacoast during the period from 2016-18. Samples were collected from the rocks with the help of hammer and chisel, packed in polythene zip wrap bag,labelled, kept in the ice box and immediately transported to the Zoology department research Laboratory of S.D.S.M. college, Palghar within an hour. Further samples were processed 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 samples were serially diluted by 10 fold serial dilution method in the normal saline solution up to 10^{-7}. The 10^{-7}dilution 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 plating following differential media simultaneously during processing of the samples, Baird Parker agar, Slanetz and Bartley agar, Macconkey agar, Violet red bile agar andThiosulphate Citrate Bile Sucrose agar.Salmonella Shigella agar and Xylose lysine deoxycholate agar was streaked after enriching the sample in selenite cystine broth at 37^0C for 18 hrs. Suspected microbes were further identified by growth pattern and morphology of the colony, direct microscopic examination and different biochemical tests (Cowan and Steel.,1993 and Hi-media Product information 1992, 2016, 2018).Authentification of representative organism was carried out at geneOmbio lab Baner, Pune.

Result

Total 12 samples of edible oyster(Crassostreagryphoides) were collected from Kelvacoast and the same were processed for Total viable count(T.V.C) and

different pathogenic bacteria.The T.V.C ranged from 1×10^7 to 35×10^7cfu/ml. Out of 12 samples processed, the total 44 isolates were isolated.Enteobacter spp. and Pseudomonas spp. Occurred in maximum number of samples (10 each) followed by Aeromonas spp. (7), Providencia spp. (5) and Bacillus spp. (4). The sample wise number is shown in Table 1and % wise pathogenic microbes in Table 2 and figure 3.

Gram Positive Rods

From the total 44 isolates isolated from the 12 edible oyster samplesBacillus flexuswere encountered in 04 samples which constituted09% of the total organisms.

Gram Negative Rods

From the total 44 isolates isolated from the 12 edible oysters samplesAeromonas spp.encounted in maximum number and constituted (29%) followed byPseudomonas spp. (25%), Enterobacterkobei(23%) andProvidenciavermicola(14%).

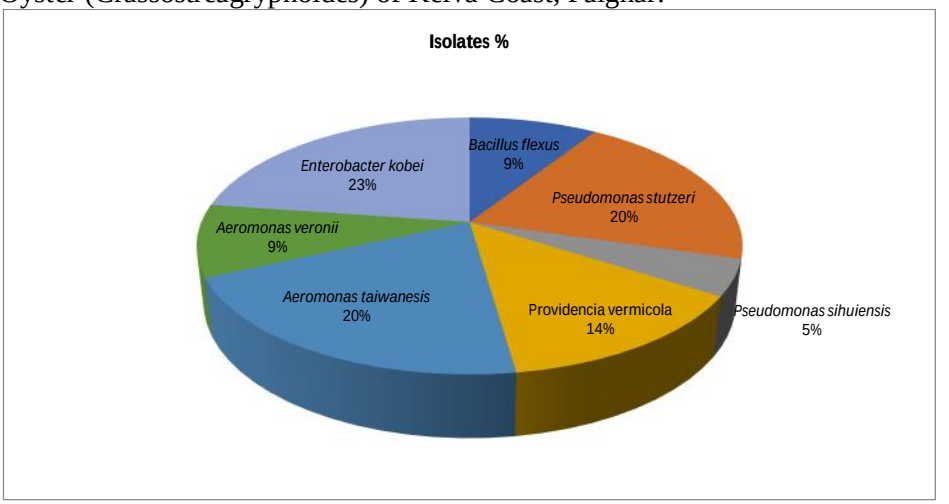
Table 1. Sample wise microbes of edible oyster (Crassostereagryphoids) from Kelva coast of Palghar

Sample No.	Name of microbes	Total No. of microbes	T.V.C=×1 0^7(cfu/ml)
1	Pseudomonas stutzeri, Providenciavermicola, Enterobacterkobei	03	10
2	Pseudomonas stutzeri, Pseudomonas sihuiensis,Providenciavermicola, Aeromonaveronii, Aeromonataiwanesis (2), Enterobacterkobei	07	20
3	Pseudomonas stutzeri, Pseudomonas sihuiensis, Bacillus flexus, Aeromonastaiwanesis, Enterobacterkobei	05	20
4	Pseudomonas stutzeri, Providenciavermicola, Bacillus flexus, Aeromonasveronii, Aeromonastaiwanesis,Enterobactereakobei	06	25
5	Providenciavermicola, Aeromonastaiwanesis, Enterobacterkobei	03	30
6	Providenciavermicola, Bacillus flexus	02	08
7	Pseudomonas stutzeri, Aeromonasveronii, Aeromonastaiwanesis, Enterobacterkobei	04	35
8	Pseudomonas stutzeri, Aeromonastaiwanesis, Enterobacterkobei	03	06
9	Pseudomonas stutzeri, Enterobacterkobei	02	05
10	Providenciavermicola, Enterobacterkobei	02	16
11	Pseudomonas stutzeri, Enterobacterkobei	02	01
12	Pseudomonas stutzeri, Bacillus flexus, Aeromonastaiwanesis(2),Aeromonasveronii	05	12
	TOTAL	44	

Table 2. Percentage of different pathogenic microbes isolated from Edible Oyster (Crassostreagryphoides) of KelvaCoast, Palghar.

Sr. No.	Name of Isolates	No. of isolates	%
1.	Bacillus flexus	04	09
2.	Pseudomonas sihuiensis	02	05
3.	Pseudomonas stutzeri	09	20
4.	Aeromonasveronii	04	09
5.	Aeromonastaiwanesis	09	20
6.	Providenciavermicola	06	14
7.	Enterobacterkobei	10	23
	G.T.	**44**	**100**

Figure 3.Diagramatic percentage of different microbes isolated from Edible Oyster (Crassostreagryphoides) of Kelva Coast, Palghar.



Discussion

Gram Positive rods

From the total 44 isolates isolated from 12 samples of edible oyster only 04 isolate were of gram positive rod Bacillus flexus, which constituted 09% of the isolates. Bacillus flexus is an aerobic, Gram-variable, rod-shaped, endosporeforming, oxidase positive bacteria. The endospores are ellipsoidal, located in central/paracentral, unswollen sporangia.These bacteria may be isolated from feces (poultry) and soil (Wikipedia).

Gram Negative rods

Gram negative rods constituted more than 90% of the total isolates. The maximum number shared by Aeromonas spp.(29%)Members of the

genusAeromonas have been associated with a wide spectrum of enteric and non-enteric diseases, in both immunocompromised and immunocompetent patients (Janda and Abbott, 1996, 1998). Aeromonasveronii was originally described by Hickman-Brenner et al. (1987) as a novel species in the genus Aeromonas that had previously been referred to by the Centers for Disease Control as Enteric Group 77.A. veroniibiovarveronii has rarely been isolated from humans and little information is available regarding its occurrence in clinical specimens and its ability to cause infections. In the original study (Hickman-Brenneret al., 1987), it was isolated from various non-sterile sites, such as wounds, faeces, sputum, maxillary sinus and endotracheal tube, in which its clinical significance remains unclear. In recent years, numerous cases of Aeromonas infection have been described in Taiwan (Huang et al., 2007; Wu et al., 2007). (Figueraset al., 2000) initiated the investigation to identify genetically a group of extra intestinal Aeromonas strains isolated in the National Cheng Kung University Hospital (Tainan, Taiwan) using a previously described 16S rRNA gene RFLP method Aeromonastaiwanensis was first described in 2010 on the basis of one strain ($A2\text{-}50^T$ = LMG 24683^T = CECT 7403^T) recovered from the wound of a hospitalized Taiwanese patient (Alperi et al., 2010).So far, one additional clinical fecal and four environmental A. taiwanensis isolates have been recorded (Senderovich et al.,2012 and Beaz-Hidalgo et al.,2012).

11 isolates of Pseudomonas spp. were recorded from 12 samples of edible oysters. Out of 11 isolates 9 isolates were Pseudomonas stutzeri and 2 were of Pseudomonas sihuiensis which constituted 20% and 5% respectively. A strain of Pseudomonas stutzeri was isolated from the leg ulcers (Lapaget al.,1968).Pseudomonas stutzeri is a Gram-negative, rod-shaped, motile, single polar flagellated, soilbacterium first isolated from human spinal fluid.(Lehmann et al.,1896 and Sijderiuset al.,1946) It is a denitrifying bacterium,(Lalucatetal.,2006) and strain KC of P. stutzeri may be used for bioremediation as it is able to degrade carbon tetrachloride(Sepulveda-Torreset al. 1999).

Pseudomonas sihuiensis is 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 spp. In the present study it is being reported and might be first ever findingin edible oyster from Kelva coast of Maharashtra, India.

10 isolates of Enterobacterkobei were recorded in the present finding which is 23% of the total isolates.HaraldHoffmannet al., 2005first time reported patient with a history of urinary bladder operation developed a urosepsis with an Enterobacter isolate displaying the E. cloacae phenotype.

Providenciavermicolarecorded six time and constituted 14% of the total isolates isolated.Providencia is a genus within the Enterobacteriaceae family closely

related to the Providencia and Morganella generaand later from a diseased rohu fish (Muderet al.,1991), but this species has not been implicated in human infection and have not been reported to cause human infection (http://www.antimicrobe.org/b227.asp).

Conclusion

This piece of study is the first attempt to highlight the occurance of pathogenic microbes from the edible oysters from kelvacoast of Palghar which in turn might be affecting the health of the sellers/consumers. Further in depth study is required on the edible oysters to link to the health hazards.

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