Section III

Research Articles and Short Communications

Screening of Edible Fish Pathogens

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Abstract : A microbial study of organisms associated with fresh fish samples was carried out. Samples from the fish skin, gills and ice water were cultured in five different media; Nutrient agar, Baird parker’s agar, Xylose Lysine Deoxycholate agar, Thiosulphate-citrate-bile salts-sucrose agar, Violet red bile agar media for the examination of *Salmonella*, *Vibrio*, *Staphylococcus, Coliforms*. The highest colony count was obtained from the skin samples in all the media. It is recommended that better handling and processing methods should be adopted to reduce or eliminate health risk to fresh fish consumers.

Keywords : Organisms, media

Introduction

The last two decades have seen appreciable increase in global fish trade and the need to enforce safety standards and regulations on imported consignment especially from developing nations fraught with unacceptable levels of microbiological contamination.

Contamination concern has been on high loads of unsuspected spoilage by microorganisms like *Salmonella sp., Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (Bramsnacs, 1999 and Gram *et al.*, 2000).Fish possess a neutral to slightly acidic pH and high moisture content which favor growth of a wide range of microbes coupled with their poikilothermic nature (Herbert *et al.*, 1997). Fish spoilage essentially can be attributed to three main factors namely; microbial, enzymatic or autolytic and chemical spoilage (oxidative rancidity) of which microbiological contamination has been noted as the main cause of fish deterioration.

Methods and Materials

(a) Sample Information

(1) Location – Vitawa goan, (Latitude19^{o}18’60”, Longitude 72^{o}99’12”8919) Thane, Maharashtra.

(2) Samples used – Mackerel (Rastrelliger kanagurta)

Bombay duck (Harpadon nehereus)

Prawns (Penaus monodon)

Ice water (in which the fishes are kept
before selling)

(3) Season - Monsoon”

(b) Collection of sample – Sample was collected from the street vendors from the fish market.

(c) Isolation and Enumeration

(1) Serial dilution - The fresh fish species were killed and macerated in a mortar and pestle,

1 gm of fish tissue was dissolved in test tube containing 9ml of sterilized distilled water to obtain a solution of 10ml.Serial dilution up to 10^{-5} was carried out on extracts from the skin and gill.

(2) Spread plate – It is the method of distributing bacteria evenly over the surface of the agar plate. 0.1 ml is spread aseptically by the sterile glass spreader until the plate is dry.

(3) Total bacterial count – Quadrant method was used to enumerate the colonies formed. Here the plate is divided into 4 parts by drawing a vertical and horizontal line. Colonies of 1 quadrant are counted and are multiplied by 4. And result is the total bacterial count of that plate.

(d) Mediums used

(1) Nutrient agar (NA) – It is the complete media used for the cultivation of non-fastidious bacteria.

(2) Baird Parker’s agar – Selective media used for growing *Staphylococcus* colonies showing a clear zone.

(3) Xylose Lysine Deoxycholate agar (XLD) – Selective growth medium for *Salmonella* and *Shigella* species.

(4) Thiosulphate-citrate-bile salts-sucrose agar (TCBS) – Selective media used to isolate Vibrio species.

(5) Violet red bile agar (VRBA) – Selective medium used for the isolation of *coliforms*.

Result and Discussion

Table 1: Table represents the number of colony forming units (cfu)/0.1ml for the water sample in which the fish are kept during marketing. Water sample is diluted in 1:1 ratio and the plates were incubated at $37^{o}C$.

Media	Water sample	
	Undiluted water sample	Diluted water sample
Nutrient agar	300	200
Baird Parker's agar	150	60
TCBS agar	54	25
XLD agar	78	25
VRBA agar	150	115

Table 2: Table represents cfu/0.1ml for the samples of body and gill from Bombay duck. Plates were incubated at $37^{o}C$ for 24 hrs.

Media	BOMBAY DUCK				
	Body surface			Gills	
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}
Nutrient agar	65	--	--	43	28
Baird Parker's agar	32	--	--	--	--
TCBS agar	--	--	--	--	--
XLD agar	--	--	--	--	--
VRBA agar	220	150	93	13	5

Table 3: Table represents cfu/0.1ml the samples of body and gill from Prawns. Plates were incubated at $37^{o}C$ for 24 hrs.

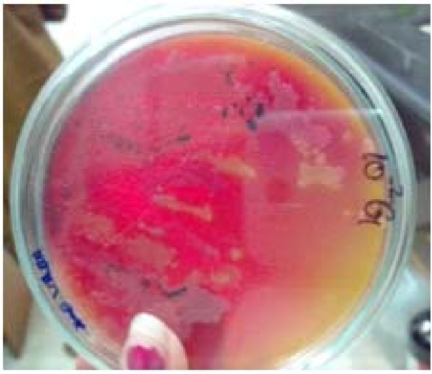
Media	PRAWNS				
	Body surface			Gills	
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}
Nutrient agar	289	53	33	66	49
Baird Parker's agar	--	--	--	--	--
TCBS agar	58	--	--	--	--
XLD agar	38	--	--	--	--
VRBA agar	116	--	4	32	--

Table 4: Table represents cfu/0.1ml the samples of body and gill from Mackerel. Plates were incubated at $37^{o}C$ for 24 hrs.

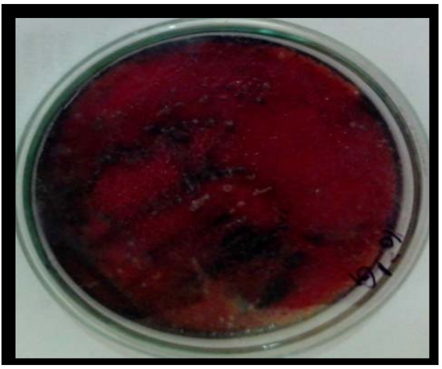
Media	MACKEREL				
	Body surface			Gills	
	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}
Nutrient agar	28	2	--	Matte	139
Baird Parker's agar	22	2	--	Matte	202
TCBS agar	42	1	--	Matte	Matte
XLD agar	--	--	--	Matte	29
VRBA agar	--	--	--	Matte	Matte

Mediums showed various changes related to their colour. TCBS agar turned its colour from green to yellow showing black colonies. Sucrose fermentation was the cause for colour change. The non Vibrio spp produced H_2S and hence the colonies appeared black; XLD agar changed its colour from red to yellow due to the degradation of xylose, lactose and sucrose. It also showed black pigmentation which are due to H_2S producing spp. It is generally accepted that fish with microbial load of >106cfu/0.1ml is unacceptable from the microbiological point of view and unfit for human consumption (Cheesbroughl, 2000).

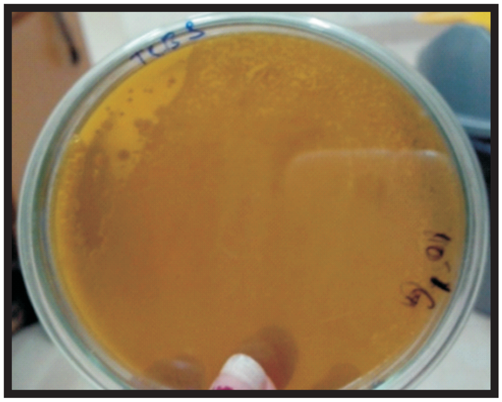
Images of media after incubation period of 24 hrs.



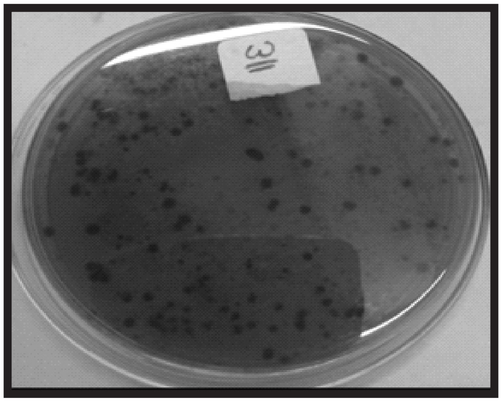
XLD agar (Black Pigmentation



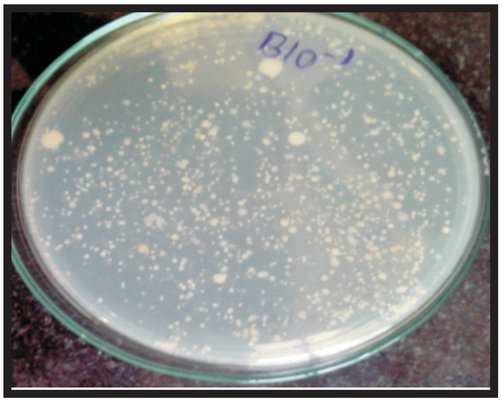
VRBA agar (Red to Yellow)



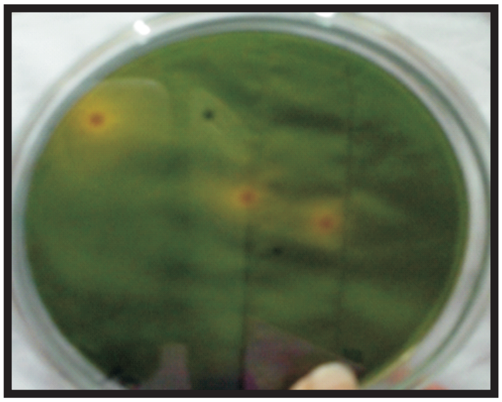
VRBA agar (isolated colonies



TCBS agar (Green to Yellow)



TCBS agar(Yellow colonies)



Nutrient agar (isolated colonies)

Conclusion

The study has revealed a moderate level of bacterial contamination of fish sold in supermarkets and by street vendors. Lack of knowledge regarding sanitary handling of food and poor processing conditions on the streets contributed to the high bacteria levels in the fish. The cutting of fish by street vendors in the absence of freely flowing water introduced more pathogens to the fish. The lack of infrastructure and use of ice at street sale point may have allowed for the growth of bacteria on the fish. These circumstances therefore, represent a potential health risk to fish eating society in the country if left unmonitored. Stringent regulations and monitoring activities coupled with food safety training of suppliers (fishermen and traders) and consumers on various aspects of Good Hygiene Practice (GHP), Good Manufacturing Practice (GMP) and Hazard Analysis and Critical Hazard points (HACCP) is strongly recommended. In view of the findings of this research work it is therefore recommended that good hygienic conditions and use of clean and free flowing water during processing should be strictly adhered to. After fishing activity, optimum storage conditions should be made available such as fresh fish should be stored at low temperatures so as to inhibit the growth of bacteria.

Future research

Based on these findings, it is important to broaden the scope of future research studies in this area like inhibition of these microorganisms by using antibiotics. According to the research paper the microbes like *Salmonella, Pseudomonas* as sensitive to antibiotics like amoxicillin, gentamycin and tetracycline (Thrower, 2000). So these antibiotics can be spread over the ice in which the fishes are stored. Different antibiotics with differing concentrations are to be tested further by using Agar cup method or Disc diffusion method. Synergistic effect of antibiotics n also be studied for better results.

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References

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Herbert *et al.*, 1997; WOGU, M.D. and MADUAKOR, C.C.; Evaluation of Microbial Spoilage of Some Aqua cultured Fresh Fish in Benin City Nigeria; Ethiopian Journal of Environmental Studies and Management(3) PP 09.

Thrower, 2000; WOGU, M.D. and MADUAKOR, C.C.; Evaluation of Microbial Spoilage of Some Aqua cultured Fresh Fish in Benin City Nigeria; Ethiopian Journal of Environmental Studies and Management(3) PP 15.