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Effects of using utility water with infectious bacteria for commercial fish storage and market selling

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ABSTRACT

Fish is one of the best protein sources available to mankind in quality as well as quantity. It is used as a staple food in countries with long coastlines and extensive inland water resources. It is a valuable source of vitamins like A, B, iodine, and oils containing polyunsaturated fatty acids. However, in all flesh food, fish is the most susceptible to tissue decomposition, rancidity development, and microbial spoilage. Fish quality is directly related to bacterial load, which itself depends upon the conditions of transport, handling, and processing. The water used for fish washing is mostly contaminated with infectious spoilage organisms and pathogens. The ice which is used to preserve fish, primarily in fishing trawlers, landing centers, and local markets, is also contaminated heavily with micro-organisms.

Keywords: *Aeromonas Hydrophilla, Pseudomonas Fluorescent, Shigella Spp., Salmonella Spp., Listeria Monocytogenes, Escherichia Coli, and Fecal Coliforms*

1. INTRODUCTION

The quality of fish continuously changes during different progress stages from harvesting to marketing. After death, the fish has to cross rigor mortis and then the body of fish acts as a suitable medium for the growth and multiplication of bacteria. The surface (scales and skin) of fish contains the highest number of bacteria because the slime layer of skin affords luxuriant medium for their growth. During life, micro-organisms are present on the external surface of fish and the gut, but the flesh is usually sterile. After death of fish, the micro-organisms diffuse into flesh and increase slowly at first, and then more rapidly causing a sequence of changes in odor and flavor. Not only bacteria and their metabolic products are responsible for spoilage, but the enzymes of the fish muscles and intestine are also involved. Activity is particularly great in fishes which have fed heavily recently due to which early rupture of gut takes place by dissemination of enzymes and micro-organisms. Bacterial infestations in fish and fish products may influence human health either directly by inducing disease or indirectly through the effect on human beings. Fish muscles and intestine are also involved. Activity is particularly great in fishes which have fed heavily recently due to which early rupture of gut takes place by dissemination of enzymes and micro-organisms. Bacterial infestations in fish and fish products may influence human health either directly by inducing disease or indirectly through the effect on human beings.

2. MATERIALS AND METHODS

Bhopal, the city of lakes, is situated at 23˚16'N latitude and 77˚26'E longitude. It possesses a number of small and large water bodies, which in addition of promoting aquaculture activities also add to the scenic beauty of the city. However, these water bodies are under great environmental stress due to pollution from various sources. Since last few decades, private entrepreneurs have been using these water bodies for the production of fish. Generally the poly-culture of Indian and exotic major carps is being practiced in these water bodies. Incidences of various health hazards have been observed in these fishes. Fishes, from these water bodies, with high microbial load reach the market where the prevailing improper handling and unhygienic conditions make them unfit for human consumption. Itwara fish market, Bittan fish piplani and govind-pura fish markets were selected for this study. These fish markets were run under the control of Bhopal Municipal Corporation.

The condition of these markets was extremely poor and unhygienic. The chicken market, situated adjacent to the fish market, aggravates this condition more. Bacterial contamination is most prevalent in this market. Water which is used for fish keeping was taken from these two markets for bacteriological examination. For the purpose of bacteriological examination, the methodology given by Bullock et al. (1971), Austin and Austin (1987) and Plumb (1994) have been followed.

3. SAMPLING METHOD

Samples for bacteriological examination were collected by taking water in different test tubes and later they were diluted serially (101,102,103). After serial dilution, the inocula were poured on different selective agars for growth, viz. Rimler-Shotts agar, Pseudomonas isolation agar, Drosete egg medium, Trypticase soy agar, Deoxychocolate agar, Columbia agar, Listeria enrichment medium, Robertson's cooked meat medium, Bismuth sulfite agar, Arabinose ammonium sulfate cholate agar, LES endo agar, Baird- Parker agar, Blood agar, Aspergillus differential medium, Malt extract agar, Potato dextrose agar, Corn meal agar and Yeast extract agar.

Incubation The temperature and time plays an important role in the incubation of bacteria for culture. The temperature ranges, over which different pathogens grow, differ considerably, but as a matter of practical expediency, a single incubation temperature within the range of 15˚C to 37˚C was generally used for isolation purpose.

4. PURIFICATION OF BACTERIA

Pure bacterial colonies were obtained by taking small inocula from mixed colonies of bacteria and aseptically streaked on selective agars with a sterile inoculating loop, incubated at 37˚C temperature for 24 hours. The colony which develops singly on a petri plate is further streaked on the agar plates till a pure colony is obtained.
Identification Gram reaction test was applied for the isolate obtained from fishes, ice, water, swab samples from market floors, fishermen's implements, fish carrying crates and swab samples from fishermen's hands. Characterization of pure culture was done by applying morphological and biochemical tests. Morphological characterization for this purpose can be illustrated by following characteristics which were taken into account:

1. Size of the colony
2. Shape of the colony
3. Color of the colony
4. Margin of the colony
5. Elevation of the colony
6. Opacity of the colony

Gram staining reaction this test determines the type (Gram positive/ Gram negative) of bacteria. For this test, small colonies were taken for smear preparation. Air dried and heat fixed smear was stained for 1 minute with crystal violet, rinsed in running tap water, then one drop of Gram's iodine solution was [19] poured over the slide for 1 minute, it was rewashed with running tap water, decolorized by alcohol - acetone solution for 8 - 10 seconds and counter stained for 30 minutes with safranine.

The smear was washed with tap water thoroughly and gently blotted dry prior to microscopic examination. Appearance of purple or violet color indicated the presence of Gram positive bacteria where as the appearance of pink or red color indicated the presence of Gram negative bacteria. Motility Nutrient agar was used for the determination of motility of bacteria. A drop of culture suspension was taken on a clean grease free slide. A cover slip was placed in such a way that an air bubble was created. The slide was immediately observed at the magnification of 40x of microscope. The creation of an air bubble made the observation of motile bacteria easy. This method is used as a convenient alternative to hanging drop method.

5. OBSERVATIONS

Heavy bacterial loads were observed in Itwara and Bittan markets followed by Piplani and Govindpura respectively

Table 1: Showing bacterial flora of water collected from four fish markets of Bhopal Bacteria

Organism	Itwara	Bittan	Piplani	Govindpura
Aeromonas hydrophila	6.0x103	5.3x103	3.0 x103	2.5 x103
	CFU/g	CFU /g	CFU /g	CFU /g
Pseudomonas fluorescens	4.0x103	6.5 x103	1.0 x103	1.0 x103
	CFU /g	CFU /g	CFU /g	CFU /g
Shigella sp.	5.5 x103	4.5 x103	0.5x103	1.0x103
	CFU /g	CFU /g	CFU /g	CFU /g
Salmonella sp.	6.5 x103	3.1 x103	0.1x103	1.0x103
	CFU/g	CFU /g	CFU /g	CFU /g
Listeria monocytogenes	10.1x103	8.1x103	4.5x103	3.5x103
	CFU/g	CFU /g	CFU /g	CFU /g
Escherichia coli	460	93	23	23
	MPN/g	MPN/g	MPN/g	MPN/g
Fecal coliforms	420	80	20	10
	MPN/g	MPN/g	MPN/g	MPN/g

6. DISCUSSION

Washing generally reduces the organic load on skin, but if the fish is washed/rewashed with the unhygienic water it leads to adherence of various pathogenic bacteria. Water samples used by fishermen collected from all the four markets of Bhopal were investigated, Aeromonas hydrophila (6.5 x 103 CFU /g), Shigella sp. (5.5 x 103 CFU /g), Salmonella sp. (6.5 x 103 CFU/g), Listeria monocytogenes (10.1 x 103 CFU/g), Escherichia coli (460 MPN/g) and Fecal coliforms (420 MPN/g) were dominant in Itwara fish market whereas Pseudomonas fluorescens (6.5 x [63] 103 CFU/g) was dominant in Bittan fish market.

This observation is in agreement with Spencer (1956, 1957) and Castell (1954) who stated that samples of water taken from commercial washing troughs have an average count of 2.2 x 106 /ml at 37˚C whereas running water wash reduces the load of market fish by 75 to 80%. On the other hand, mechanical jet washing reduced the load on an average of 95%. Georgala (1957a, 1958) stated that after filleting, the average skin counts were 103-6 at 37˚C, 106-8 at 20˚C and 104-7 at 0˚C /cm2 which is slightly higher than the averages for the whole fish after washing 102 at 37˚C, 104 at 20˚C and 104 at 0˚C / cm2. The subsequent increase is due to the result of contact with the filleting benches which are the most important sources of fillet contamination. Spencer (1959) and Kreuzer (1954) reported that market containers contain hundred millions of bacteria per square inch even after application of water from high pressure jet even the old boxes contain thousands of bacteria per square inch.

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