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Microbiology of Hands of Fishermen from Four Major Fish Markets of Bhopal

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***Abstract*: Microbial analysis of hands of fishermen at different stages from catching to selling point up to consumer level is important for assessing the quality of fish. Bacterial load of hands of fishermen collected from different fish markets of Bhopal was determined. Samples collected from Itwara and Bittan market contained considerably more bacteria than those collected from Piplani and Govindpura market. Besides, sample from close roofed markets contained more bacteria than from open market. The possible reason for this variation might be due to fact that the sunlight and washing of hands frequently after gutting of fish reduces the bacterial load. Among the different parts of fish body, maximum number of bacteria was found in scale and skin followed by gill, gut and muscle. High number of coliform bacteria was also found in the samples tested. Proper handling and precautions is important for maintaining the quality.**

***Keywords:* Fishermen, Bacterial load, coliform, Handling.**

I. INTRODUCTION

Fish meat has excellent nutritional value being rich in proteins, vitamins and unsaturated fatty acids. It is also extremely perishable and the safe consumption requires adequate sanitary conditions from the moment of catch, through preparation, sale and consumption. Various factors pose a condition of risk to fish food safety and they range from contamination from the environment where it is caught up to contamination by the consumer before eating.

II. MATERIALS AND METHODS

Bacteriological examination

Swab Samples for bacteriological examination of hands of fishermen collected from four major fish markets of Bhopal were collected with the help of swab sticks. These swabs were diluted serially (10^1,10^2,10^3). 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.

Incubation

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

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.

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Identification

Gram reaction test was applied for the isolate obtained from fishermen's hands. Characterization of pure culture was done by applying morphological and biochemical tests.

Morphological characterization

For this purpose, following characteristics were taken into account

- Size of the colony
- Shape of the colony
- Colour of the colony
- Margin of the colony
- Elevation of the colony
- 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 poured over the slide for 1 minute, it was rewashed with running tap water, decolourized 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 colour indicated the presence of Gram positive bacteria where as the appearance of pink or red colour 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.

III. BIOCHEMICAL TESTS

Oxidation/fermentation test

This test is related with the production of acid from glucose metabolism under aerobic conditions in the basal medium of Huge and Leif son (1953). The appearance of yellow colour, after 1, 2 and 7 days incubation was taken as an indication of oxidation reaction. In case, the yellow colour does not appear, in stipulated time, it was taken as an indication of fermentation reaction.

Oxidase test

For this test, a piece of filter paper was placed in a petri dish and moistened with freshly prepared cold 1% w/v of tetramethyle-p-phenylenediamine dihydrocholoride solution. Subsequently, a bacterial colony was smeared over the moistened paper by means of platinum loop. Appearance of dark purple colour within 30 seconds indicated positive reaction.

Catalase test

For this test, young colony were scraped with the help of glass rod and transferred to a drop of hydrogen peroxide on glass slide. Appearance of effervescence within 1 minute indicated positive reaction.

In dole production test

For this test, 1% w/v peptone water was inoculated with bacteria and incubated for 7 days and then a few drops of Kovac's reagent were added to the peptone water. Appearance of pink or cherry red colour yielded positive reaction.

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Hydrogen sulphide production test

For this test, 1% w/v peptone water was inoculated with bacteria and incubated for 7 days and then a lead acetate paper strip was inserted between the plug and the tube. Appearance of black colour on paper after 4 to 7 days indicated positive reaction.

Methyl red and Voges Proskauer (MR-VP) test

For methyl red test, bacteria were inoculated on methyl red medium and incubated at 30˚C for 2 to 5 days. Then 2 drops of methyl red solution were added to it. Appearance of strong red colour after one hour indicated positive reaction. It was later confirmed by Voges Proskauer test by adding 0.6 ml of napthol solution and 0.2ml of 40% KOH aqueous solution which gave strong red colour after one hour, indicated positive reaction.

Decarboxylase test

For this test, arginine, lysine, ornithine and a control (arginine) were inoculated with bacterial colony and overlayed it with mineral oil, then tubes was incubated at 35˚C temperature for four days. The inoculated media were examined daily. The media first became yellow due to acid production from glucose and later on they changed to violet due to decarboxylation. The control tube remained yellow.

Urease production test

For this test, urease medium was inoculated with bacteria and incubated for 28 days. Appearance of red colour indicated positive reaction.

Coagulase test

For this test, B-P agar was inoculated with bacteria and incubated at 37˚C temperature. The coagulation of human or animal plasma in absence of calcium indicated positive reaction.

Starch hydrolysis test

For this test, Starch agar was inoculated with bacteria and incubated at 30˚C temperature for 5 days. Then the inoculated petri plate was flooded with lugol's iodine solution. Appearance of clear colourless zone indicated positive reaction while blue colour indicated negative reaction.

IV. RESULTS AND DISCUSSION

Samples have been taken by swabbing the hands of fishermen for investigating their bacterial load. It was observed that Streptococcus iniae (10.5×10^3 CFU/g), Staphylococcus aureus (6.9×10^3 CFU/g), Shigella sp. (10.1×10^3 CFU/g) and Salmonella sp. (10.1×10^3 CFU/g) were dominant in the hands swab of fishermen from Itwara fish market whereas Pseudomonas fluorescens (3.5×10^3 CFU/g) was found dominant in the hands swab of fishermen from Bittan fish market. Samples collected from Itwara fish market was observed to contain more no. of bacteria than other three fish markets. This could be because Itwara fish market is roofed and poorly constructed. It is situated at the center of the city where it is surrounded by chicken and beef market. Other fish markets are fully aerated under the open sky.

Fig 1: Showing sample collection of fishermen's hands.

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Table – 1: Showing bacterial flora of hand samples of fishermen collected from four markets of Bhopal

Bacteria	Itwara	Bittan	Piplani	Govindpura
Pseudomonas fluorescens	$1.4x10^3$ CFU/g	$3.5x10^3$ CFU/g	$0.3x10^3$ CFU/g	$0.6x10^3$ CFU/g
Streptococcus iniae	$10.5 x10^3$ CFU/g	$10.3 x10^3$ CFU/g	$1.5 x10^3$ CFU/g	$1.0 x10^3$ CFU/g
Staphylococcus aureus	$6.9 x10^3$ CFU/g	$2.6 x10^3$ CFU/g	$1.0 x10^3$ CFU/g	$0. 3 x10^3$ CFU/g
Shigella sp.	$10.1 x10^3$ CFU/g	$7.1x10^3$ CFU/g	$2.5 x10^3$ CFU/g	$3.0x10^3$ CFU/g
Salmonella sp.	$10.1 x10^3$ CFU/g	$5.0x10^3$ CFU/g	$1.5 x10^3$ CFU/g	$1.5x10^3$ CFU/g

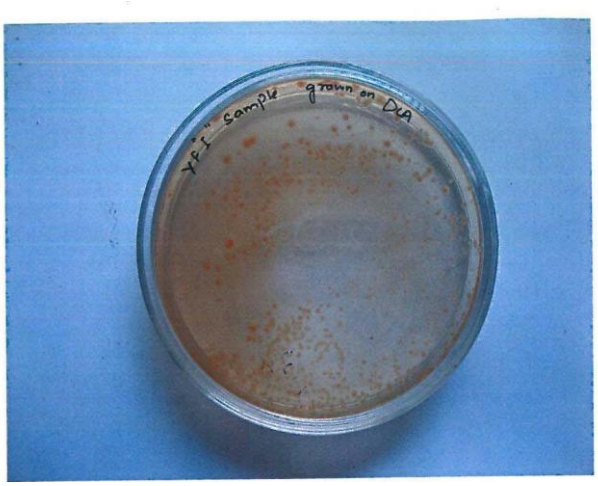

Fig-2: Growth of Shigella

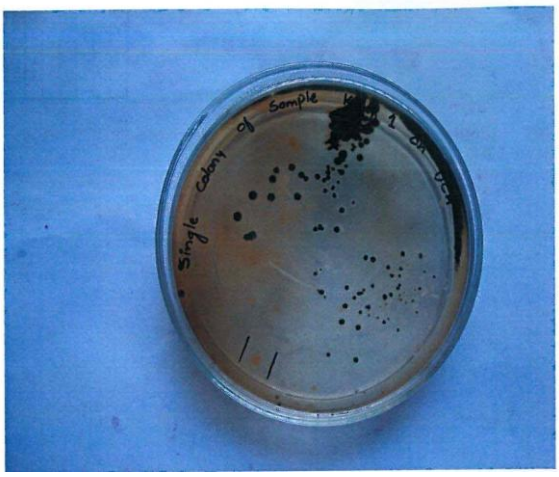

Fig-3 Growth of Salmonella

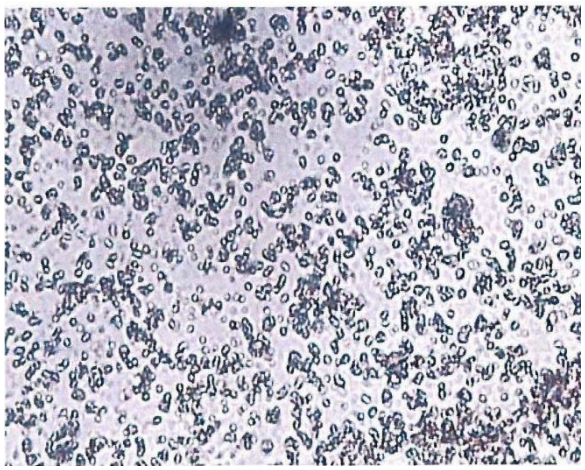

Fig4: Microscopic Veiw of Staptoccous Spp.

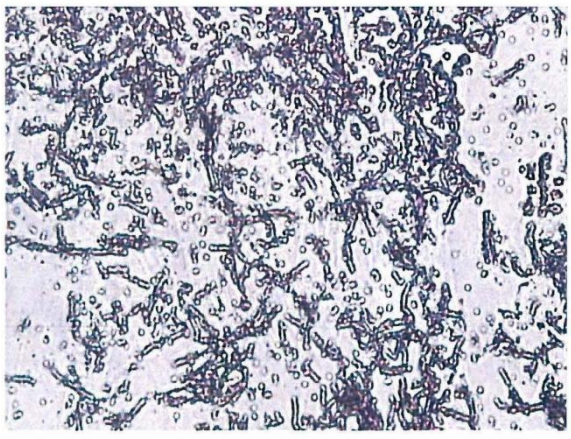

Fig-5 Microscopic View of Staphyloccous Spp

This observation is in conformity with Jayasinghe, P. S. (2006), Jha, R. K. (2003), Kitao, T. (1993), Klinger, I. (2001), Koh, H.T., Kurup, A and Chen, J. (2004), Lalitha, K. V. and Surenderan, P. K. (2003), Lalitha, K. V. and Iyer, K.M.(1990),

Lerke, P., Farber, L. and Adams, R. (1967), Levin, R. E. (1968),Lior, L. Y., Baron, F. and Gautier, M. (2003), Liston, J., Stansby, M. E. and Olcott, H. S. (1976) ,Spencer, R. (1956-59). who observed that fishes contained bacterial load from the moment of catch to the consumer through unhygienic handling.

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