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Microbial Examination of Mackerel (*Rastrelliger kanagurta*) from the Satpati ice factory, Palghar, Maharashtra, India.

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Abstract: The present study was carried out for microbial examination of fish mackerel from satpati ice factory. The study was performed during the period from December 2016 to May 2019. 12 samples of *Rastrelliger kanagurta* were processed and a total of 23 isolates were isolated which were represented by 8 veritas of microbes. The TVC ranged from 08 x 10^{-7} to 145x10^{-7} cfu/gm. The dominance of *Escherichia coli* and *Vibrio spp.* was observed followed by *Staphylococcus spp*., *Enterococcus spp.* and *Bacillus spp.*, and *Enterobacter spp.* and *Proteus spp*. The findings of this study infer that the fish obtained from those sources contain potentially pathogenic microorganism. It was also observed that the poor sanitary conditions prevailed in the ice factory are crucial for the microbial contamination of fishes. This paper is attempted to elaborate pathogenic microbes of mackerel and sanitary conditions prevailing in satpati ice factory

Key words: -Fish Microbiology, TVC, *Rastrelliger kanagurta*, Satpati.

1. INTRODUCTION:

Fish and fishery products are not only nutritionally important but also important in global trade as foreign exchange earner for a number of countries in the world (Yagouband and Ahmed 2003). About 80% of animal protein in our diet comes from fish alone (Rubbi *et. al*., 1978). However, consumption of fish may sometimes cause disease due to infection or intoxication. It is believed to be the reflection of the general contamination in the aquatic environment. The true incidence of diseases transmitted by fish is display tray, uniform of salesmen, electric balance and usually unknown source in the vicinity. It has been estimated that as few as 1% of the diseases are actual cases of food borne disease (Chowdhury and Baqluis 1997).

Fish are of great concern for export earnings because of their higher nutritive value such as high protein content, with little or no carbohydrate and fat value. But fish may be contaminated at various stages of transport, handling, and processing. This contamination may be related to the raw materials, personnel, and processing tools such as forklifts through leakage, insect, and pest harborage. Additionally, seafood can become contaminated during storage and processing (Bryan 1980 and Gangarosa *et. al*., 1968).

Transmission of the pathogens can be through the food or the handling of the fish. There have been great economic losses reported due to food borne illness such as dysentery and diarrhea resulting from consumption of contaminated fish. The microbial association with fish compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic and / or pathogenic in nature (Mhango *et. al*., 2010). It is reported that most iced fish in the major marketing centers of India are of substandard quality (Nair *et. al.,* 1974 and Govindan, 1985). Subburaj *et. al.*, (1984) stated that the market premises and market floor and the water could be major sources of contamination of fish in the fish markets in Mangalore, India.

In the present study, investigation was carried out on the human pathogenic bacteria of mackerel (*Rastrelliger kanagurta)* stored in the ice factory of Satpati from where large amount of fishes are supplied to the market and hotels of Mumbai in particular.

2. MATERIALS AND METHODS :

Satpati is one of the biggest fishing village on the coast of Arabian sea India. It is about 90 km north of Mumbai, located in the Palghar Taluka of Palghar district in Maharashtra. The main industry in Satpati is fishing, with large exports abroad.

Mackerel (*Rastrelliger kanagurta*) samples were collected on monthly basis and seasonal availability for microbial studies from Satpati, ice factory from December 2016 to May 2019. The samples were collected in sterile polythene bags and transported to the laboratory of Zoology, S.D.S.M. College, Palghar in thermo-cool containers for further processing.

The whole body of Mackerel (*Rastrelliger* kanagurta) was used as sample and 10 grams was transferred to a sterile beaker to which 90 ml of sterile 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 TVC on plate count agar (PCA). The agar plates were inoculated by pour plate method and incubated at 37 ^{0}C for 24 hrs. The 10^{-4} dilutions of each sample 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, Salmonella Shigella agar, TCBS agar and Bacillus cereus agar. 0.1 ml of the 10^{-4} dilutions were inoculated by the spread plate method on the above media plates and were incubated at 37 ^{0}C / 44 ^{0}C for 24 / 48 hrs. 1gm of fish sample was taken in 9 ml of Selenite cystine broth which was incubated at 37 ^{0}C for 18 hrs. It was then streaked on Salmonella Shigella agar and incubated at 37 ^{0}C for 24 hrs. for *Salmonella* species. The colonies from the differential media plates were transferred in sterile peptone water and the same were identified by morphology, Gram's staining and biochemical tests. The preparation was carried out according to Cowan and Steel (1970, 1993) and Diliello (1982) and Hi-media (2013, 2015). The representative isolates were verified by Gene Ombio Technologies PVT. LTD, Pune.

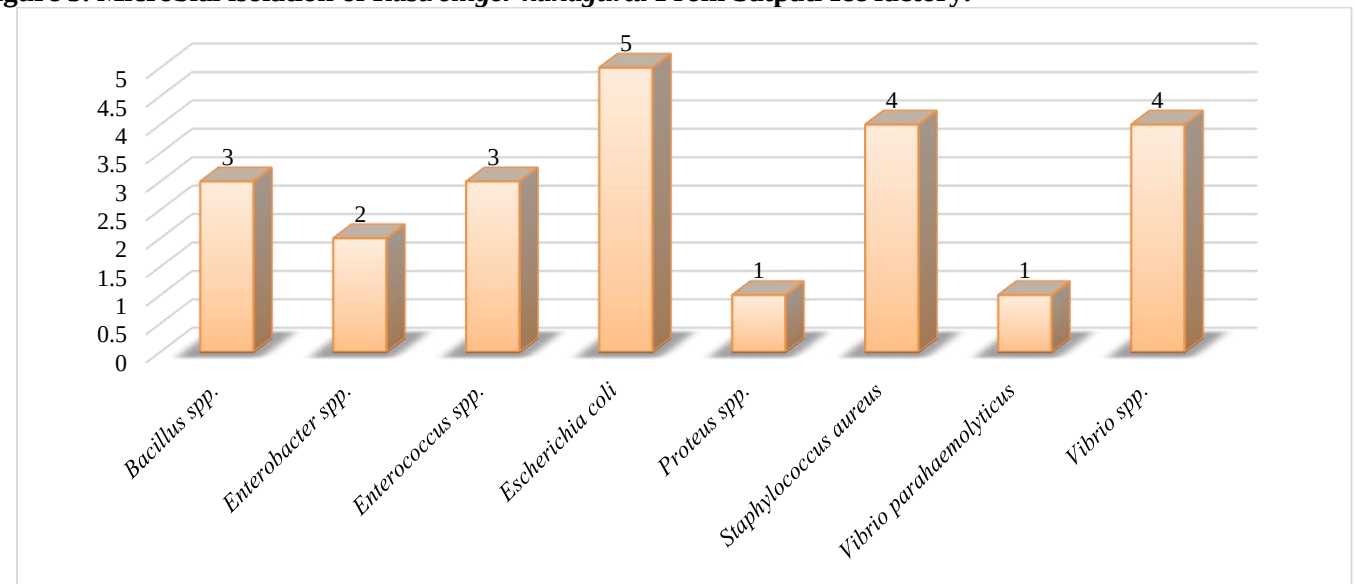
3. RESULT: -

In the present study 12 samples of mackerel (*Rastrelliger kanagurta)* from Satpati ice factor were examined for microbial quality. A total of 23 isolates were isolated and the same were represented by 08 types of microbes. The TVC ranged from 08 x 10^{-7} to 145 x 10^{-7} cfu/gm. The dominance of *Escherichia coli* and *Vibrio spp.* (05 each) was observed followed by *Staphylococcus spp*. (04), *Enterococcus spp.* and *Bacillus spp.* (03 each), *Enterobacter spp.* (02) and *Proteus spp.* (01). The prevalence of *Vibrio* spp. was represented by *Vibrio parahaemolyticus* and *Vibrio spp.*, Table 1 and Figure 1.

Table 04. Qualitative microbial isolation of *Rastrelliger kanagurta* from Satpati ice factory.

Sr. No.	Isolates	No of isolates	Percentage
1.	*Bacillus spp.*	03	13.04
2.	*Enterobacter spp.*	02	8.70
3.	*Enterococcus spp.*	03	13.04
4.	*Escherichia coli*	05	21.74
5.	*Proteus spp.*	01	4.35
6.	*Staphylococcus aureus*	04	17.39
7.	*Vibrio parahaemolyticus*	01	4.35
8.	*Vibrio spp.*	04	17.39
	Total	**23**	100.00

Figure 3. Microbial isolation of *Rastrelliger kanagurta* From Satpati Ice factory.



4. DISCUSSION:

Incident of these microbes can be responsible to human infections. *Vibrio* spp. capable to causing gastrointestinal illness (*gastroenteritis*). Several species are pathogenic for marine vertebrates and invertebrates (Holt *et. al.,* 1994). Three species, *V. cholerae, V. parahaemolyticus, and V. vulnificus*, are well-documented human pathogens. *Vibrio* species account for a significant proportion of human infections from the consumption of raw or undercooked shellfish (Kaysner and DePaola, 2004). *V. parahaemolyticus* is a marine / estuarine bacterium causing gastroenteritis in humans through consumption of seafood.

Most *Escherichia coli* strains are harmless, but some can cause serious food poisoning. Kumar *et. al.,* 2005, reported that estuaries and coastal water bodies, which are the major sources of seafood in India, are often contaminated by human activities and are associated with the widespread occurrence of *Escherichia coli* in seafood. *Escherichia coli* is often used as an indicator for fecal contamination; it can cause human illness, some strains of *Escherichia coli* are capable of causing food-borne disease, ranging from mild enteritis to serious illness and death. It can cause a variety of diseases, including diarrhea, dysentery, hemolytic uremic syndrome, and bladder and kidney infections. Different strains are usually associated with different diseases; this versatility of *Escherichia coli* strains is due to the fact that different strains have acquired different sets of virulence genes (Teophilo *et. al.,* 2002).

Prevalence of *Staphylococcus aureus* was observed during the present study. *Staphylococcus aureus***,** it is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most *Staphylococcus* infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections. Enterotoxins produced by *Staphylococcus aureus* are another serious cause of gastroenteritis after consumption of fish and related products. In 08 of 10 samples of fish, counts of *Staphylococcus aureus* were detected than permitted by Brazilian legislation (Vieira *et. al.,* 2001).

Members of the genus *Bacillus* have been isolated from a wide variety of aquatic and terrestrial environments. Some *Bacillus* genus is pathogenic and some are in harmless in nature.

Enterococci have been considered a low-virulent pathogen, during the last decades they have become an important cause of a variety of infections that primarily affect debilitated and immunocompromised patients and are mainly hospital-acquired or healthcare associated infections. (Pallares and Grau 2017). *Enterococci* are important inhabitants of human and animal intestine. They are the predominant flora of the intestine in the first 2 to 3 days of life in many animals (Devriese *et. al*., 1991). Species of *Enterobacter* was also isolated during this study and are clearly opportunistic pathogens and rarely cause disease in the otherwise healthy individual (Sanders and Sanders ,1997). During this study though the presence of *Proteus spp.* was found in least amount but it is the human opportunistic pathogens and can cause urinary tract and wound infections, bladder and kidney stones and nosocomial infections.

It is indigenous to the marine environment and its survival is affected by temperature, not surviving in seawater below 10 C (Desmarchelier 1997). Seawater temperatures in Greece are ideal for survival and growth of *Vibrio parahaemolyticus* Davies *et. al.* (2001). It was found in various fishes from Greece (anchovy, bogue, mackerel, mussels, picarel, mullet), in relatively high incidence (14%), only second to that of Portuguese fish (35%)

Infants from Sweden and Pakistan were assessed for *Enterobacteriaceae* based on mode of delivery (vaginal versus cesarean) and breastfeeding behaviour (Adlerberth *et. al.*,1991). Cesarean births in Pakistan were associated with *Proteus* species colonization within 3 days, with 11 of 21 Cesarean delivered and 1 of 9 vaginally delivered infants being positive for *Proteus* spp. (P = 0.049) (Adlerberth *et. al.*,1991)

5. CONCLUSION:

In the present study, different types of pathogenic bacteria were encountered in the fish from Satpati ice factory. Human bacterial pathogen were isolated from the Mackerel *Rastrelliger kanagurta*, which are a serious threat to the fish consuming community. Unhygienic fish handling practices of these infected fishes and inadequate cooking may further contribute to the spread of these pathogens. Based on the present of pathogenic bacteria in the present study and prevalence of the unhygienic conditions at satpati ice factory, there should be healthy practice for fish storing, handling and supply to the selling women of the market. Therefore, regular more study should be conducted and published in order to establish data with comparative epidemiological and statistical values.

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