Assessment of Hygiene Status of Retail Fish Market using Seasonal Changes in Microbial Load as a Tool

Arup Kumar Das and Armaan U. Muzaddadi*
College of Fisheries, Central Agricultural University, Lembucherra, Tripura
ICAR-Central Institute of Post-Harvest Engineering and Technology, Ludhiana - 141 004, India

Abstract

The hygienic status of the Battala retail fish market Agartala, Tripura, India was assessed by microbiological analysis of Total Plate Count (TPC), Total Coliform Count (TCC), Total Psychrophilic Count (TPSC) and Total Staphylococcus Count (TSC) from the fish skin swabs, retailers' hand swabs, water, ice, selling platform swabs and cutter swabs. The presence of coliforms and Staphylococcus in higher counts in the samples was an indication of contamination from human source. The average TPC ranged from 3.82 to 6.59 log cfu cm^{-2} ml^{-1} in fish skin, ice, water and other contact surfaces (selling platforms, retailers' hand swabs and cutter) and was found above acceptable limit in almost all samples which was clearly a reflection of prevailing unhygienic condition of the market. Psychrophilic bacteria count (5.07-6.77 log cfu cm^{-2} or ml^{-1}) was significantly high (p<0.05) in fish skin and ice which may be due to the prolonged storage of fish in ice, the use of poor quality water for ice manufacturing and contamination from fish and ice box during transportation. Total coliform count in fish skin, wash water, ice and all contact surfaces ranges from 3.72 to 5.21 log cfu cm^{-2} or ml^{-1} during the study period. Unacceptable level of coliforms in all samples is indicative of the contamination with faecal matter and with the enteric pathogens as well. *Staphylococcus* sp count (3.77-5.38 log cfu cm^{-2} or ml^{-1}) for all the samples exceeded the acceptable limit which indicated the poor personal hygiene maintained among the retailers. So the results call for improvements in general infrastructure of this fish market to maintain good sanitation and hygiene.

Introduction

Tripura is a hilly and landlocked state of the northeastern region of India. Fish is favourite food of 95% people of the state with per capita consumption of 13 kg which is much higher than all over India (9 kg). This results in huge demand in the state. Such high demand is met mainly through importing fish from other Indian states or even from neighboring Bangladesh which are further marketed through the fish markets situated at Agartala, the capital city of the state. Battala Retail Fish Market is one such market which handles more than 60% of the fish sold at Agartala (Anon, 2008). Since the hygiene aspects of the market have much importance in respect of public health significance, this scientific study was conducted to evaluate the present status of the market. Food hygiene includes thorough processing and protection of food from contamination risks (Hobbs & Roberts, 1990) and if conditions are unhygienic, food contamination occurs (Marriot, 1994). Microbial contamination is the most serious form and can result in food spoilage, food poisoning or even death (Sprenger, 1993). It is desirable to determine the microbial and nutritional quality of fish reaching market in order to assure health and hygiene to the consumers (Lilabati & Vishwanath, 1996). The consumption of contaminated fish may cause various clinical disorders in human beings and has great public health significance (Beri et al., 1998). These pathogens come from various sources such as water, feed, pond, soil, bird droppings and other live forms of the surrounding ecosystems. Even the fish handlers can act as source of pathogens like *Listeria monocytogens*, *Escherichia coli* (Jaysekaran et al., 2003). Most of the studies on fish market have established that a sizable portion of the fish available in the market for consumption is not meeting the

quality criteria prescribed by Indian Standards hygiene (Nambiar & Iyer, 1990; Lakshmanan et al., 2002).

Material and Methods

The studies on the hygienic status of the Battala retail fish market including infrastructure facilities and management controls were done through survey of the market and interviewing the retailers and market authority and subsequently by micro-biological analysis. The samples were taken from fish skin, contact surface (table top), retailers' hand and cutter by swabbing. Duplicate swabs were obtained with pre-moistened sterile swabs by swabbing the standard sample area of 10×10 cm^2. For other samples such as washing water and ice, 25 ml of water and 25 g of ice were collected aseptically in sterile polythene bags. Total 36 samples, 6 samples from each source (fish skin, ice, washing water, cutting platform, cutter and hands of seller) were collected at monthly intervals starting from the month of September up to February, 2012-2013. Samplings were done twice in each week (with total 6 replica from each source), serially on first, second and third week of the month and all the data of a particular for a particular month were further pooled to have an average value for the respective month. The samples were kept in ice-box and carried to the Microbiology Laboratory of Department of Fish Processing Technology, College of Fisheries, Tripura and analysed for total plate count (TPC) by spread plate technique as per APHA (2001), Psychrophilic bacteria count, total coliform count (TCC) and *Staphylococcus* spp through the methods given by Varma (2002). Exactly 25 g of sample (for skin and ice) was introduced aseptically in sterile stomach bag (Seward stomach BA6141CPG standard bags) and macerated for 2 min with 90 ml of sterile diluents of 0.85% sodium chloride (NaCl) using stomacher (Seward Stomacher 400 Circulator, England), serial dilutions of 10^{-2}, 10^{-3} and 10^{-4} were made using 9 ml physiological saline (0.85% NaCl). Swabs were decimally diluted mixing each swab separately with 10 ml sterile saline solutions and each was mixed well using magnetic stirrer. From all the diluted samples 0.1 ml of inoculums was spread plated onto Soybean Casein Digest Agar (SCDA, HIMEDIA) using sterile glass spreader. The plates were incubated at 37°C for 36 h for TPC at same temperature for overnight for TCC. For *Staphylococcus* count, similar plating was done on to Baird Parker (BP) Agar and Vogel-Johnson Agar

plates and incubated at 37°C for 48 h. The black colonies with convex 1.0-1.5 mm in diameter having narrow white entire margin and surrounded by a mark of clearing of 2.0-2.5 mm in width were considered positive for Staphylococcus. The plates containing 25-250 colonies were counted. Staphylo-coccus colonies were purified onto Tryptose soya agar (TSA) plates and then subjected to Coagulase test by Khanna (2002). The sanitary status were mentioned after interviewing all the retailers (30) using a questionnaire, the answers as YES (+) or NO (-) with at least 90% similarity have been represented as (+) or (-).

Statistical analysis of data was performed using SPSS-16.0 (SPSS Inc., Chicago, IL, USA) for windows. Results were presented as mean (S E). A probability level of 0.05 was used to find out the significance in cases.

Result and Discussion

The Battala fish market covered an area of 0.48 ha with 40 whole sellers and 71 retailers. The primary observations clearly indicate that the sanitary condition of Battala fish market was not adequate wherein only 6 concrete platform, 4 hand pumps, 4 cleaners, 1 toilet and 8 waste bins were observed (Table 1). Being unaware of basic rules of food hygiene and sanitation, the fish handlers do unsanitary practices in market place and their personal hygiene was very poor, as they had no

Table 1. General managerial aspects of the Battala retail fish market

Particulars	Details
Area coverage	3 kani/ 0.48 ha
Market association	Battala Matsya Arokdaar Samiti, Battala Bazar
Scale of operation	Large
Working hour	Daily from 6 am to 6 pm
No. whole seller (Arokdaar)	40
No. of Retailer	71
No. of Concrete platform	6
Hand pump/supply water	4
No. of Cleaner/sweeper	4
No. of toilets	1
Waste Disposal facility	8 waste bin

regular washing of cloths, hand-washing with soap and monthly medical check-up (Table 2). Although they had daily bath, they did not use bath soap regularly. The facilities for preservation of fishes were not up to the mark, because there were no ice

Table 2. The sanitary status of fish retailers' personal hygiene of Battala fish market

Activities	Status*
Daily bath	+
Daily washing clothing	-
Hand-washing with soap after using washroom	-
Monthly medical check-up	-
Foot wash before sitting on fish display & selling platform	-

* Status mentioned after interviewing all the retailers (30) using a questionnaire, the answers as YES (+) or NO (-) with at least 90% similarity have been represented as (+) or (-).

Table 3. Overview of the sanitary properties in Battala retail fish market

Sanitary properties	Status
Changing fish-wash-water in bucket at every hour	-
Chilled storage facility	-
Chlorination of water	-
Drainage system	-
Foot dip with chlorinated water	-
Ice supply sufficient	-
Insect protectors	-
Proper fish waste disposal facility	-
Proper road facility with covered drainage system	-
Regular washing of cutter (Dao)	+
Selling platform made of concrete	+
Separate area for fish dressing	-
Sufficient toilet facilities	-
Washing and cleaning of platform alternate days	-
Washing and cleaning of platform everyday	+

* Status mentioned after interviewing all the retailers (30) using a questionnaire, the answers as YES (+) or NO (-) with at least 90% similarity have been represented as (+) or (-).

plant in the vicinity and also no cold store for the storage of unsold fish. The floors of the fish market were filthy with no proper drainage system for water and also no hygienic disposal system of wastes. There were poor appropriate infrastructure facilities for fish washing, storage, chilling and no supply of good quality water and ice (Table 3).

The bacterial load on fish skin, water, ice and contact surfaces usually reflect the general sanitary condition of fish market. The TPC is widely accepted tool to find the aerobic bacterial count in fish and other objects. Fish skin, ice, water, contact surfaces (selling platform and cutter) and retailers' hand are the possible routes of contamination while retailing. For microbiological analysis, samples were collected from three pre-identified retail Fish-stalls from each source. The mean value (log cfu cm^{-2} or ml^{-1}) of the each parameter obtained from the stalls were considered as representative of the entire market.

The average TPC of fish skin was found to be in high (5.36 to 6.43) and showed significant change (p<0.05) in all the months of sampling (Fig. 1). The counts were high during November and December months indicating higher level of contamination, cross contamination and maintenance of hygiene in the market. This period, being the peak period of harvesting season, has an ambient temperature around 30°C and this provide a favourable environment for the bacterial growth when fish were stored under un-iced condition. The findings of Sahu et al. (2008) in the domestic fish trade of Mumbai and some other previous studies involving samples from Bombay (Iyer et al., 1986) and Mangalore (Jadhav & Magar, 1970) underscore the present study. Similarly Ahmad et al. (2013) reported 7.15, 6.92 and 6.62 log10 cfu cm^{-2} in beef, sheep and goat meat respectively in retail market in Lahore. The average TCC in fish skin study period was ranging from 4.58 to 5.02 and the counts did not significantly changed (p<0.05) in different sampling months except September and February (Fig. 1).

This might be indicative of the higher level of contamination during September and February as well as impact of environmental condition. In September, the highest TCC was recorded which might be due to the high ambient temperature (mean temperature 28.5°C and humidity 83%) in that month compare to other sampling months. In addition, large amount of fish are harvested from fresh water bodies such as pond, beel and lakes in
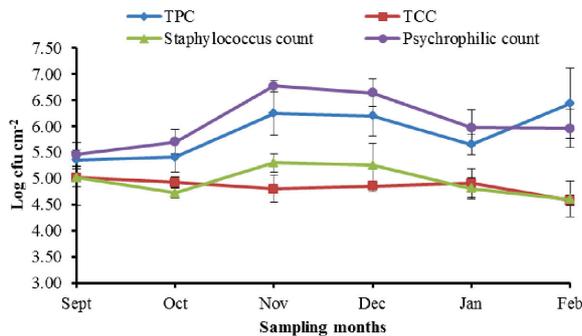
Fig. 1. TPC, TCC, *Staphylococcus* sp and Psychrophilic bacteria count in fish skin of Battala retail fish market during the study period (values are mean ± SD, n=3).

September. Hence, there is more influx of fish into the market during this period resulting in management problems in handling large amount of fish. In a similar study Marandi (2013) recorded bacterial load in the skin of Anabas as 19.2×10^6 cfu g^{-1} and in Clarias 19.2×10^6 cfu g^{-1} caught from water bodies in Odisha. However, the counts were relatively on higher side than the acceptable limit and fluctuated in all the sampling months. Shewan (1971) noted that coliform counts of freshly caught fish were relatively low, but increased considerably during handling. In retail market the raw material or products undergo many handling operation due to the long distribution chain which could be a possible reason for high coliform load. Use of poor quality ice and water during handling and transportation might be another reason for these higher counts. This observation resembles with the findings of Sudhi (2002) in fish of Kochi market (Kerala). Since, there were no separate areas for dressing the whole fish in Battala market; contamination might take place in dressing areas. The findings of Bordoloi (2010) about bacterial load in rohu fish from Agartala market also agree with the present findings. Average Staphylococcus count in fish skin swab was ranging from 4.61 to 5.30 did not show significant change (p>0.05) in different months of sampling. (Fig. 1). *Staphylococcus* sp is not found in normal micro flora of fish. The natural habitat for *staphylococcus* sp is the skin and mucus membranes of animal and man. The presence of Staphylococcus in fish is an indication of post-harvest contamination. The incidence of *Staphylococcus* sp in fishery products within limits is not a serious problem, but careless handling during processing results in the multiplication of the organism, which may lead to food poisoning (Lilabati & Vishwanath, 1996). It was noticed that the overall hygienic condition and personal hygiene maintained in the Battala retail fish market is not up to the mark. All the retailers did not wash their hand properly with disinfectant and soaps; except some species, the retailers simply dumped the fish on selling platform directly and handled them unhygienically. Kakatkar et al. (2010) reported the incidence of *Staphylococcus* sp in fishes of Domestic market of Mumbai. Papadopoulou et al. (2007) reported that the origin of *S. aureus* is the handlers rather than fish. In the present study psychrophilic bacteria were found in the range from 5.46 to 6.77 (Fig. 1). In the chilled storage, the psychrophilic bacteria may have found better growth environment due to the fish slime which can act as good nutrient source. The prolonged storage ice-storage also could be a major reason where psychrophilic bacteria proliferated slowly and dominated the mesophilic bacteria, as low temperature favoured their growth (Lakshmanan et al., 2002).

The average TPC of ice samples varied between 3.82 and 5.60 and significantly differed (p<0.05) among all the months of sampling. The psychrophilic bacteria count for ice ranged from 5.07 to 5.89 (Fig. 2). Perhaps the containers holding the ice might have been contaminated by previous fish (Vieira, 1997). The ice were crushed and simply dumped in the floor of the mill and same ice was used for icing fish in fish box. The average TCC of ice varied between 3.98 and 4.85 and did not show significant change (p>0.05) in different sampling months except in September (Fig. 2).
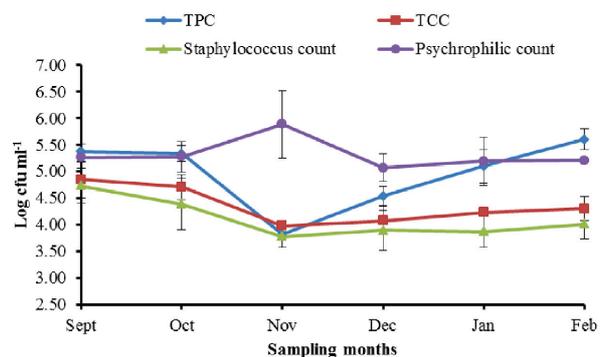

Fig. 2. TPC, TCC, *Staphylococcus* sp and Psychrophilic bacteria count in ice samples of Battala retail fish market during the study period (values are mean ± SD, n=3).

The observations of present study also resemble with the findings of Hassan et al. (2011) in ice used in fish carrying crates from certain fish market of Bhopal. The average TPC of water samples were ranging from 4.52 to 6.56 and showed significant change ($p<0.05$) in all the months of sampling, whereas, the TCC was ranging from 4.12 to 5.01 and the values did not significantly change ($p>0.05$) in different sampling months (Fig. 3). Repeated use of same water for cleaning and washing purposes which became contaminated due to the fish slime, blood and wash residual of retailers' hand perhaps resulted in high TPC and TCC. High coliform counts in water of reservoir of Nigeria were also reported by Atiribom & Kolndadacha (2014). Nicolas et al. (2006) in dish washing waters from street food processing sites in Ouagadougou showed similar observations. *E. coli* is an indication of faecal pollution and is considered as an indication of the presence of other pathogenic bacteria (Jay, 1992). Ice and water samples showed *Staphylococcus* counts from 3.77 to 4.73 and 4.20 to 5.38 in ice and water respectively which significantly changed ($p<0.05$) in different sampling months (Fig. 2 and 3). Cross contamination from fish, fish boxes, retailers hand perhaps the possible reason for the high count in ice and water. The findings of present study substantiate the findings of Vieira et al. (1997) in market of Brazil.
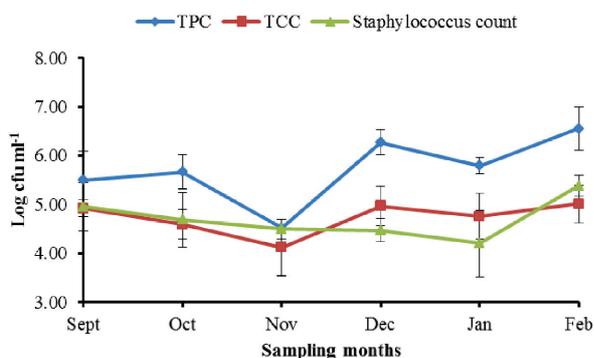
Fig. 3. TPC, TCC and *Staphylococcus* sp count in water samples of Battala retail fish market during the study period (values are mean ± SD, n=3).

The selling platform showed bacterial counts 4.96 to 6.59, 4.09 to 5.21 and 4.00 to 5.05 for TPC, TCC and Staphylococcus respectively with significant differences ($p<0.05$) in different sampling months (Fig. 4).
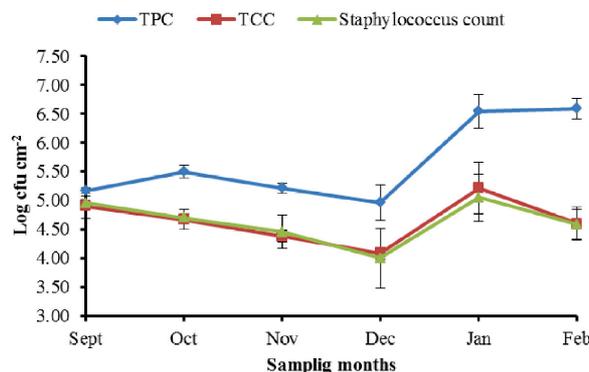
Fig. 4. TPC, TCC and *Staphylococcus* sp count in swab samples of selling platform of Battala retail fish market during the study period (values are mean ± SD, n=3).

These findings is similar to the findings of Mol & Saglam (2004) in the market Turkey (Fasanmi et al., 2010) from meat sellers tables in various markets of Ibadan, Nigeria. They concluded that the high microbial load obtained from the butchers table is an indication of the ineffectiveness of the method used in cleaning the tables, which are usually washed with water only. Similarly improver washing and cleaning methods of selling platforms were the possible reasons for higher microbial load in swab samples of selling platforms. The cutter surface also showed TPC, TCC and Staphylococcal count (SC) from 4.30 to 5.36, 4.03 to 4.79 and 4.08 and 4.90 respectively and also showed significant changes ($p<0.05$) during all sampling months (Fig. 5). The cutters were not washed immediately between the operations; instead they were cleaned by wiping with bear hand and this may be one of the major reasons for such high counts. The higher coliform counts and Staphylococcal counts indicate poor personal hygiene of the fish handlers. The present study is also supported by the study of Mackey & Derrick (1979) that isolated bacteria of common types from cutting knives. Aerobic Colony Count (ACC) $<10^5$ cfu g^{-1} was regarded as marginal limits of acceptable microbiological quality for food based on standard methods of International Commission on Microbiological Specifications for Foods (Okareh & Erhahon, 2015). Hence the counts were marginally above the limit.

Retailer's hand swabs showed a TPC from 4.59 to 5.13 with insignificant variations ($p>0.05$) during the sampling months (Fig. 6). The TCC and average staphylococcal counts were ranging from 3.72 to 4.63
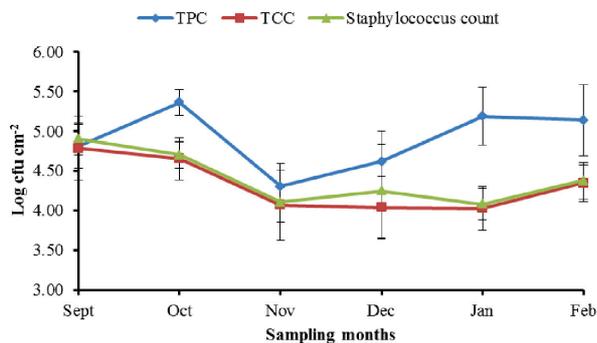
Fig. 5. TPC, TCC and *Staphylococcus* spp. count in swab samples of cutter of Battala retail fish market during the study period (values are mean ± SD, n=3).

and 4.14 to 5.03 respectively which changed significantly ($p<0.05$) in different months of sampling (Fig. 6). Primary observations recorded poor personal hygiene (Table 2) and no use of disinfectant and soaps for washing hands. Other observations include washing hands by dipping hand in dirty waters which was also used for cleaning of fish, weighing balance etc. and cleaning hands by wiping hands with a piece of cloth. Studies done by Reij et al. (2004) attributed poor hygiene, particularly deficient or absence of hand washing as the causative mode of transmission. The findings of Nicolas et al. (2006) investigated in the markets of Ouagadougou recorded almost similar values and observed similar causes for the higher microbial load in hand swab. Higher bacterial contamination of the hands of food handlers as indicator of hand washing efficacy in convenient food industries in South Africa was given by Lamberchts et al. (2014). They reported highest bacterial count from the hand samples as 7.4×10^3 cfu cm^{-2} which exceeded the legal limits.
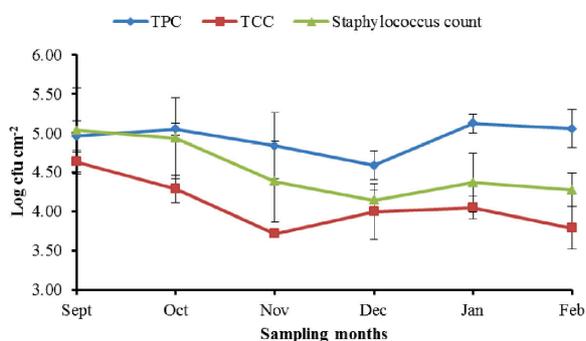

Fig. 6. TPC, TCC and *Staphylococcus* sp count in hand swab of retailers of Battala retail fish market during the study period (values are mean ± SD, n=3)

Study of hygiene status of fish markets and the fish retailers can provide information which may be important for policy matters in regards to taking necessary steps for improvement by the concerned authorities. Ali et al. (2016) studied the occurrence of *enteric* bacteria in food handlers and surfaces of food manufacturing industry in Pakistan which also have concurrence with the present study. They determined overall isolates percent prevalence for *Enterobacteriaceae* as 47%, Coliforms as 31% and *Escherichia coli* as 22% in all the samples and reported majority of the floor surfaces were highly contaminated, where washing and sanitation practices were inappropriate. Being a very important market in the state capital of Tripura, Battala fish market needs immediate attention for hygienic improvement of the market. This study specifically underscores steps to be taken up by the authorities like initiate awareness, sensitization and skill development programmes for the fish handlers on hygienic and scientific fish handling, storage and primary processing; re-construct the drainage and waste disposal systems with appropriate orientation taking expert consultation; establish proper system of water supply, water treatment, pest control measures, floor tiling, toilets & washrooms, effluent treatment plant, cold storage and ice plants and appropriately design and construct the Whole-sale Fish Market with truck entry, delivery, auction place, storage etc. keeping sufficient distance from the retail market.

Acknowledgements

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