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Evaluation of microbial quality of raw fish meat sold at fish meat outlets of Bikaner

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Abstract
Fish are among one of the major sources of food for many countries globally and a vital source of protein. The microbiological analysis of raw fish meat sold at fish meat outlets of Bikaner were assessedin relation to public health significance. In the present study, a total of 60 raw fish samples were collected from the city areas of Bikaner, Rajasthan. Raw fish meat samples were analyzed for the existence of pathogenic bacteria *E. coli* and *S. aureus* through the conventional cultural techniques. The mean total viable and psychrophilic count for them was 6.2x 10^6 cfu/gm and8.38 x 10^4 cfu/gm, respectively. As per ICMSF (1986), 25 (41%), 21 (35%) and 14 (23%) samples were found to be fit for consumption, marginally acceptable and rejected, respectively. Out of 60 raw fish samples, 29 (48.33%) samples were positive for *E. coli* and 20 (33.33%) samples were positive for *S. aureus.*

Keywords: fish, *E. coli*, *S. aureus*, microiological analysis

Introduction
Fish is a vital source of food for people globally. Around 60 per cent of the developing countries derive 30 per cent of their annual protein from fish (Abisoye *et al*., 2011) [1]. It is the most important source of high quality protein, providing approximately 16 per cent of the animal protein consumed by the world's population. Fish has become an increasingly important source of protein and other elements necessary for the maintenance of healthy body and constitute an important food component for a large section of the world population. The quality of fish meat is the major concern to the food processors, consumers and public health authorities (FAO, 1997) [13].

Fish has high consumer preference due to its inherent nutritive value, taste and easy digestibility other essential nutrients and omega 3-fatty acids and its low fat content as compared to other meats (Rhea, 2009; Pal and Das, 2010) [28, 26].

The microbial association with fish compromises safety and the quality for human consumption; particularly critical is when the micro-organisms are opportunistic or pathogenic in nature (Mhango *et al*., 2010) [21]. However, fish are
susceptible to a wide variety of bacterial pathogens, most of which are capable of causing disease and are considered by some to be saprophytic in nature (Petronillah *et al*., 2013) [27].

The pathogenic strains of *E. coli* may cause diarrhoea by producing and releasing toxins (called enterotoxigenic *E. coli* or ETEC) and cause of food borne illness in fish (Lee and Marks, 2009) [19].

The high isolation rate of *S. aureus* indicates poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of utensils and working surfaces most staphylococci occur as commensally; however, *S. aureus* strains producing various toxins and enzymes are responsible for diseases in animals and humans. The pathogenic properties of *S. aureus* are mainly due to various virulence factor such as protein A, clumping factor, coagulase, fiberonectin, heamolysin, nucleases, exfoliative toxin and enterotoxins (Foumier, 2008) [14].

Most outbreaks of food poisoning associated with fish and seafood derive from the consumption of raw or insufficiently heat treatment, insufficient cooking and cross-contamination during processing (Mohammed *et al*., 2017) [5, 23]. Fish sold at open market and exposed to ambient temperature increases likelihood of spoilage. Marketing and handling chain involving fresh fish is important from public health point of view (Kapute *et al*., 2012) [17].

Materials and Methods

The present research work was conducted in the Department of Veterinary Public Health and Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, Bikaner Rajasthan, University of Veterinary and Animal Sciences, Bikaner, India.

A total of 60 raw fish samples were collected from various retail outlets of Bikaner city. All the precautions were taken to collect samples aseptically, avoiding external contamination. About 10-20 grams of fish samples were taken in sterilized test tubes and immediately brought to the laboratory. The samples were processed within 4-6 hours of collection.

Ten grams fish from each sample was triturated with 90 ml sterile normal saline solution and poured in a sterilized test tube using sterile filter paper.

The Total viable count (TVC) test for each raw fish sample was done using pour plate method as per the American Public Health Association (APHA) (1992) [6].

For total viable count, duplicate petri plates were inoculated with 1 ml each of the inoculum from the various tenfold dilutions of the samples. About 10-15 ml of sterilized molten total plate count agar media maintained at 45- 50 ºC was poured in each petri plates and it was mixed with inoculum by rotating 2-5 times each by giving clockwise and anti-clockwise movement. The medium was then allowed to solidify. The inoculated plates were incubated at 37 ºC for 48 hrs. The plates showing 30 to 300 colonies of bacteria were selected for counting in a colony counter and the counts were expressed as colony forming units (cfu)/gm of sample.

Method used for psychrophilic count of fish samples is similar as described earlier in total viable count method but the inoculated plates are incubated at 10 ºC for 7-10 days.

Isolation and identification of bacteria

The fish samples were subjected to aerobic cultivation. Each sample was streaked on nutrient agar plates in primary, secondary and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hr at 37 ºC. After 24 hr of incubation, these isolated colonies were cultured on eosine methylene blue agar (EMB) plates, mannitol salt agar (MSA) plates for isolation of *Escherichia*, *Staphylococcus* and incubated for 24 hours at 37 ºC. The growth was examined for the colonial morphology and pigmentation and in order to obtain pure culture different types of colonies were sub-cultured on separate nutrient agar plates.

Results and Discussion

According to ICMSF (1986) [16] the raw fish meat having total viable counts less than 5.0 x 10^5 cfu/gm is acceptable, between 5.0 x 10^5 cfu/gm to 5.0 x 10^6 cfu/gm is marginally acceptable and more than 5.0 x 10^6 cfu/gm should be rejected. So as per ICMSF (1986) [16], 25 (41%), 21 (35%) and 14 (23%) samples were found to be fit for consumption, marginally acceptable and rejected, respectively.(Table -1 and Figure – 1)

Table 1: Total viable counts of raw fish samples according to ICMSF (1986) [16]

Range of TVC/gm of fish	Grade	No (%) of raw fish samples collected from various retail meat outlets
< 5.0 x 10^5	Acceptable	25 (41%)
>5.0 x 10^5 to <5.0 x 10^6	Marginally Acceptable	21(35%)
>5.0 x 10^6	Rejected	14(23%)
Total No.		60

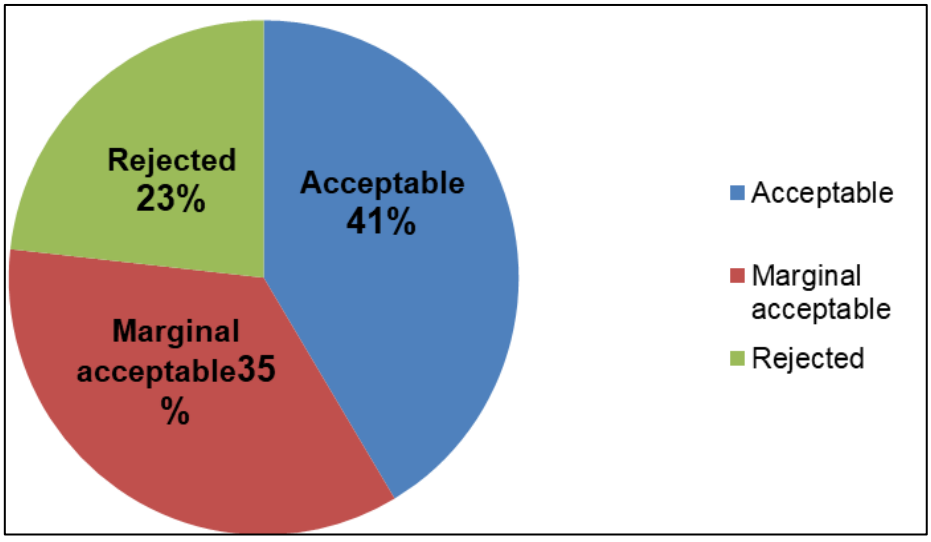

Fig 1: Categorization of raw fish samples according to ICMSF (1986) [16]

In the present study, the mean total viable count (TVC) for all the 60 raw fish samples was found to be 6.2 x 10^6 cfu/gm. Dutta *et al.* (2010) [12] reported total bacterial load from four different ponds and found 5.6 × 10^6 cfu/gm, 4.3 × 10^6 cfu/gm, 3.2 × 10^6 cfu/gm, 1.0 × 10^6 cfu/gm, respectively which is almost similar to our present investigation. Das *et al.* (2007) [10] found 5.1 x 10^6 cfu/gm total bacterial loads in Batashi (*Clupisoma atherinoides*) which is nearly similar to present values. Mohamed and El-Mossalami. (2009) [22] found total viable count as 5.4 x 10^5 cfu/gm and 5.8 x 10^5 cfu/gm in Rohu fish (*Labeo rohita*) which is lower with the present investigation. Whereas Rokibul *et al.* (2013) [29] reported6.7 × 10^9 cfu/gm which was higher values than present investigation.

The findings of the present study indicate that the raw fish marketed in this area has high Total viable count. In local

markets, different kinds of fish are kept together and the sellers do not maintain proper hygiene which creates the possibilities to come in contact with several pathogens and the sellers uses ice, mostly prepared from contaminated water, to preserve fish after being caught which may be a potential source of contamination. Therefore, aseptic handling, proper storage condition and maintaining homeostasis during lag between fishing and marketing claim an appropriate practice of hygiene for ensuring the microbiological quality of fish as well the consumer safety. It is worth to note that the pathogens existing in the contaminated foods may harbour virulence genes which might be responsible for disease outbreaks (Noor *et al*., 2013) [25].

In present investigation the mean total psychrophilic count (TPC) for all the 60 fish samples was 8.38 x 10^4 cfu/gm. Kose and Erdem (2004) [18]; Alparslan *et al*. (2013) [3] and Aman *et al*. (2017) [4, 5], reported psychrophilic count 2.29 x 10^4,2 x 10^4 and 1.55 × 10^4 cfu/gm, respectively which is lower than the present study. While, Mol *et al*. (2007) [24] reported 1 x 10^6 cfu/gm and 2.4 x 10^5 values which is also higher than that of present results. Amany *et al.* (2017) [4, 5] reported psychrophilic count 9.7 x 10^5 ± 2.2 x 10^5 cfu/gm for Mullet which is also higher than that of present results.

In the present investigation, out of 60 raw fish samples 29 (48.33%) samples were positive for *E. coli*(Figure -2) while 20 (33.33%) were for *S. aureus* (figure-3).Samaha and Hendawy (2017) [31] reported 42.00% *E. coli* from different markets at Alexandria province which is nearly similar to the present study. Bujjamma and Padmavathi (2015) [9] isolated 24.47% *S. aureus* in fish samples from domestic fish market of Guntur, Andhra Pradesh which is lower than present findings. Gupta *et al*. (2013) [15] observed 20.8% and 29.34% fish samples positive for *E. coli* which is lower than that of present study. Meiyarasi *et al*. (2017) [20] reported the prevalence rate of *E. coli* from fresh raw fish samples to be 14.28% which is also lower than that of present study. Atwa (2017) [8] found lower values for both as 25% fish samples positive for *E. coli* and 12.5% for *S. aureus.* Dutta and Sengupta (2016) [11] also isolated 65.00% *E. coli* in the fish samples from Kolkata whereas, Ali (2014) [2] reported 100.00% prevalence of *S. aureus* which is very much higher than present findings. Arfatahery *et al*. (2016) [7] confirmed 34.30% prevalence of *S. aureus* in fish samples which are similar to the present findings whereas, Rong *et al*. (2017) [30] reported 37.2% prevalence of *S. aureus* in fish samples which is slightly higher than present investigation.

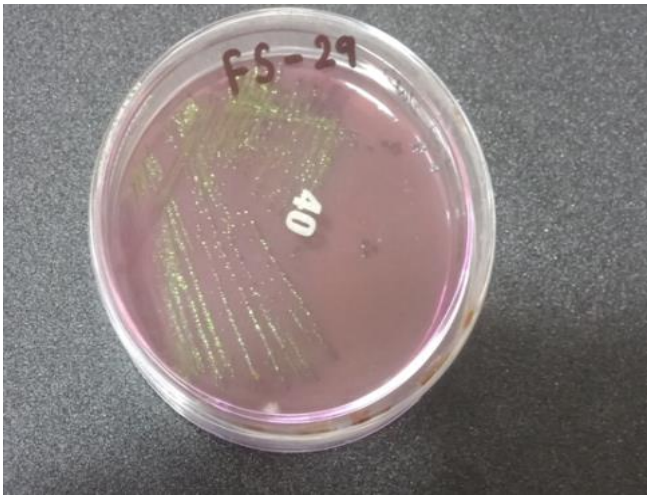

Fig 2: Isolation of *E. coli* on EMB agar from fish sample

Fig 3: Isolation of *S. aureus* on mannitol salt agar from fish sample

Conclusion

Raw fish have been recognized as a major carrier of foodborne pathogens of viz. *Escherichia coli* and *Staphylococcus aureus*. It would therefore be recommended that the local fish sellers should be familiarized with the Good Hygienic Practice (GHP) and HACCP for better services. These fish sellers play significant role by giving hygiene raw fish and fish products to people and maintaining their general health so they can give quality food to public necessary for their good health.

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