The Pharma Innovation Journal 2022; SP-11(7): 1324-1327

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 1324-1327

www.thepharmajournal.com
Received: 18-03-2022
Accepted: 11-06-2022

Devender Choudhary
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Chandani Jawa
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Deepika Dhuria
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Manohar Sain
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Maya Mehara
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Manoj Kumar Kalwaniya
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Corresponding Author
Devender Choudhary
Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Prevalence of *Staphylococcus aureus* in raw fish meat samples and assessment of the coagulase test in the identification of *Staphylococcus aureus* isolates

Devender Choudhary, Chandani Jawa, Deepika Dhuria, Manohar Sain, Maya Mehara and Manoj Kumar Kalwaniya

Abstract
Raw fish naturally have a number of bacteria, and this can be opportunistic and causing foodborne infections rapidly. *Staphylococcus aureus* are one of the major bacterial agents causing food borne diseases in humans worldwide. The present study was conducted to determine the prevalence of *Staphylococcus aureus* in raw fish. A total of 60 raw fish samples were collected from the city areas of Bikaner, Rajasthan. The isolation and identification were done according to standard bacteriological analytical methods. The mean total psychrophilic count for them was 8.38 x 10^4 cfu/gm. Out of 60 raw fish samples, 20 (33.33%) samples were positive for *S. aureus.* Coagulase production was recorded in 18 (90.0%) *S. aureus* isolates whereas, 2 isolates (10.0%) were coagulase negative.

Keywords: *S. aureus*, fish, psychrophilic count, coagulase test

Introduction
Fish has high consumer preference due to its inherent nutritive value, taste and easy digestibility. It is one of the most important sources of animal protein available in the tropics and has widely been accepted as a good source of protein and other elements for maintenance of healthy body (Andrew, 2001) [7]. The genus Staphylococcus comprises several species, of which *S. aureus* is one of the major bacterial agents causing food borne diseases in humans worldwide (EFSA, 2010; Le-Loir *et al*., 2003) [12, 18]. Staphylococcus species are one of the most important food borne opportunistic bacteria which isolated from fish samples and some of Staphylococcus species are Potential pathogens and the high population of these bacteria indicates the general quality of fish and the degree of the spoilage it might have undergone (Albuquerque *et al*., 2007) [2]. 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) [19]. Staphylococci are usually differentiated into two groups using the coagulase test. It is assumed that coagulase positive staphylococci (CoPS) are usually pathogenic, even when in some cases they can cause asymptomatic colonization in healthy individuals, and coagulase negative (CoNS) are saprophytic or cause opportunistic infections (Gonzalez-Martín *et al*., 2020) [15].
The distribution of multidrug resistant of *Staphylococcus* species increased in last years and act as etiological infection agent responsible for food poisoning and representing a risk to health when they are enterotoxogenic strains and responsible for significant levels of morbidity and mortality in human (Albuquerque *et al*., 2007) [2]. 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].

Material Methods
The present research work was conducted in the Department of Veterinary Public Health and Epidemiology, 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 for the present study. 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.

Preparation of inoculum

Ten grams fish from each full raw fish sample was triturated with 90 ml sterile normal saline solution and poured in a sterilized test tube using sterile filter paper. Tenfold serial dilutions were made using sterile normal saline solution with dilution ranging from 10^{-1} to 10^{-6} in the test tubes.

Psychrophilic count

For Psychrophilic 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 45-50ºC 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 are incubated at 10 ºC for 7-10 days. 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.

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 mannitol salt agar (MSA) plates for isolation of 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.

Test for coagulase production

Coagulase enzyme production by *Staphylococcus aureus* is considered a criterion for determining its pathogenicity and is evaluated by coagulase test. 10 ml human blood was collected aseptically in a test tube containing EDTA @ 1mg/ml. The blood was centrifuged at 2500 rpm for 15 minutes. The clear plasma obtained was then transferred into another sterilised test tube for use in the test.

The plasma was diluted ten folds (1:10) in physiological saline solution and 0.5 ml of reconstituted plasma was taken in three serological tubes. 0.1 ml of an overnight broth culture of the *Staphylococcus aureus* was added to one tube and 0.1 ml of broth culture of *Staphylococcus epidermidis* was added to second tube (negative control) and remaining third (uninoculated tube) was kept as control. The tubes were rotated gently for mixing of the contents and thereafter, incubated in water bath at 37ºC. The tubes were examined at 1, 3 and 5 hours by slowly tilting the tube at 90º angle and compared to the control tube. Clotting of plasma within 2-5 hours was recorded as a positive for "free" coagulase enzyme (Cowan and Steel, 1975) [11].

Result and Discussion

In present investigation the mean total psychrophilic count (TPC) for all the 60 raw fish samples was 8.38 x 10^4 cfu/gm. Kose and Erdem (2004) [16]; Alparslan *et al*. (2013) [4] and Aman *et al*. (2017) [5], reported psychrophilic count 2.29 x 10^4, 2 x 10^4 and 1.55 × 10^4 cfu/gm, repectively which is lower than the present study. While, Mol *et al*. (2007) [20] reported 1 x 10^6 cfu/gm and 2.4 x 10^5 values which is higher than that of present results. Amany *et al.* (2017) [6] 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 study out of 60 raw fish samples 20 (33.33%) samples were positive for *S. aureus*. Bujjamma and Padmavathi (2015) [10] isolated 24.47% *S. aureus* in fish samples from domestic fish market of Guntur, Andhra Pradesh which is lower than present findings. Ali (2014) [3] reported 100.00% prevalence of *S. aureus* which is very much higher than present findings. Rong *et al.* (2017) [21] reported 37.2% prevalence of *S. aureus* in fish samples which is slightly higher than present investigation. Arfatahery *et al.* (2016) [9] confirmed 34.30% prevalence of *S. aureus* in fish samples which are similar to the present findings.

Table 1: Details of *S. aureus* samples for coagulase test

Total no. of raw fish samples	No. of samples positive for *S. aureus*	No. of coagulase positive *S. aureus*samples
60	20	18 (90.0%)

In the present study, all the 20 isolates of *S. aureus*, isolates from raw fish samples were subjected to tube coagulase test using human plasma for production of coagulase. Among these 20 *S. aureus* isolates, 18 (90.0%) isolates were found coagulase positive, whereas, 2 isolates (10.0%) were coagulase negative (Table-1 and Figure-3) and most of the isolates coagulated the plasma completely within 2- 5 hours of incubation. Sivaraman *et al*. (2016) [22] reported 11.52% coagulase positive staphylococci in retail outlets of Gujarat which is much lower than that of present study. Field and Smith (1945) [13] studied 124 strains of staphylococci and observed the all pathogenic strains coagulase positive while nonpathogenic strains were found to be coagulase negative. The results of Kumar *et al.* (2016) [17] showed 15.78% samples positive for coagulase positive staphylococci, were lower than that of present study while Aragon-Alegro *et al*. (2007) [8] observed 54% coagulase positive *S. aureus* in raw fish samples which were lower than that of present investigation. Abdeen *et al.* (2021) [1] observed the coagulase positive Staphylococcus was 84% and 76% in beef luncheon and karish cheese, respectively which is nearly similar with the present study.

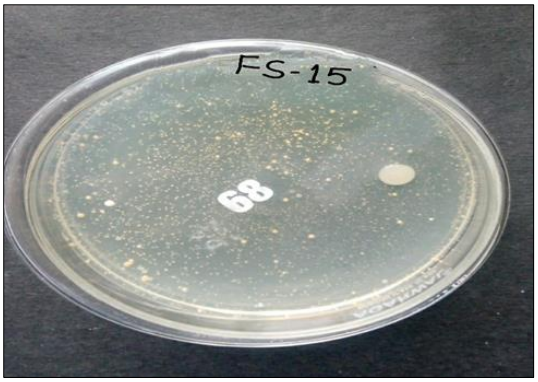

Fig 1: Total Psychrophilic Count of raw fish sample

Fig 2: Isolation of *S. aureus* on mannitol salt agar from fish sample

Fig 3: Clotting of plasma in positive coagulase test

Conclusion

Bacteria may infect the fish from outside during care less handling of landed fish. Among major external sources of bacterial contamination are ice and salt used for preservation and cross contamination during processing. Improvements in handling and processing and good sanitation and hygienic conditions at landing centres and domestic markets are urgently needed to minimize fish contamination.

The use of the coagulase test will help to identify the pathogenic and non-pathogenic strains of *S. aureus* and this test also reduce the cost of bacteriological examination.

References

1. Abdeen EE, Mousa WS, Abdelsalam SY, Heikal HS, Shawish RR, Nooruzzaman M, Abdeen A. Prevalence and characterization of coagulase positive staphylococci from food products and human specimens in Egypt. Antibiotics. 2021;10(1):75.
2. Albuquerque WF, Macrae A, Sousa OV, Vierira GHF, Vieira RHF. Multiple drug resistant *Staphylococcus aureus* strain isolated from a fish market and from fish handlers. Brazilian Journal of Microbiology. 2007;38:131-134.
3. Ali HH. Isolation and identification of Staphylococcus bacteria from fish of fresh water and its antibiotic sensitivity in Mosul city. Journal of Veterinary Research. 2014;1(1):33-42.
4. Alparslan Y, Yapici HH, Metin C, Baygar T. Determination of meat quality of sea bass (*Dicentrarchus labra*) sold at different selling areas. Emirates journal of food and agriculture. 2013;26(3):36-39.
5. Aman IM, El-Sayed Y, Ali Moustafa NY, Hamza AA. Quality assessment of Tilapia nilotica and Mugil cephalus fish from Egypt. Journal of Veterinary Medicine and Allied Sciences. 2017;1(2):1-6.
6. Amany E Mohammed, Hassan A Abdallah, Abd El-Hafez M Abd El-Hafez, Amin A, Mohamed M, Mousa. Quality assessment of some retailed marine fish and shellfish in alexandria province. Alexandria Journal of Veterinary Sciences. 2017;52(1):166-172.
7. Andrew AE. Fish processing technology. University of IIorin press, Nigeria, 2001, 7-8.
8. Aragon-Alegro LC, Konta EM, Suzuki K, Silva MG, Junior AF, Rail R, *et al*. Occurrence of coagulase positive *Staphylococcus* in various food products commercialized in Botucatu, SP, Brazil and detection of toxins from food and isolated strains. Food Control. 2007;18:630-634.
9. Arfatahery N Davoodabadi, Abedimohtasab T. Characterization of toxin genes and antimicrobial sensitivity of *Staphylococcus aureus* isolates in fishery products in Iran. Scientific Reports. 2016;6:1-7.
10. Bujjamma P, Padmavathi P. Prevalence of *Staphylococcus aureus* in fish samples of local domestic fish market. International Journal of Current Microbiology and Applied Science. 2015;4(5):427-433.
11. Cowan ST, Steel KJ. Cowan and Steel's Manual for the identification of medical bacteria. 2nd ed. Cambridge University Press, Cambridge, 1975, 1-217.
12. EFSA. European Centre for Disease Prevention and Control, The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2008. EFSA J. 2010;8(1):1496.
13. Field HIS, Smith HW. The coagulase test for staphylococci. Journal of Comparative Pathology. 1945;55:63-69.
14. Foumier B. Global regulators of *Staphylococcus aureus* virulence genes. Staphylococcus molecular genetics, 2008, 131-183.
15. Gonzalez-Martin M, Corbera JA, Suarez-Bonnet A, Tejedor-Junco MT. Virulence factors in coagulase-positive staphylococci of veterinary interest other than *Staphylococcus aureus*. Veterinary Quarterly. 2020;40(1):118-131.
16. Kose S, Erdem ME. An investigation of quality changes in anchovy (*Engraulis encrasicolus*, L. 1758) stored at different temperatures. Turkish Journal of Veterinary and Animal Sciences. 2004;28(3):575-582.
17. Kumar LR, Kasim AK, Lekshmi M, Nayak BB, Kumar S. Incidence of methicillin-resistant staphylococci in fresh seafood. Advances in Microbiology. 2016;6(06):399.
18. Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genetics and molecular research: GMR. 2003;2(1):63-76.

19. Mohammed AE, Abdallah HA, Abd El-Hafez AEM, Amin A, Mousa MM. Quality assessment of some retailed marine fish and shellfish in alexandria province. Alexandria Journal of Veterinary Sciences. 2017;52(1):166-172.
20. Mol S, Erkan N, Ucok D, Tosun YS. Effect of psychrophilic bacteria to estimate fish quality. Journal of muscle foods. 2007;18(1):120-128.
21. Rong D, Wu Q, Xu M, Zhang J, Yu S. Prevalence, virulence genes, antimicrobial sensitivity, and genetic diversity of *Staphylococcus aureus* from retail aquatic products in China. Front Microbiology. 2017;8:714.
22. Sivaraman GK, Lalitha KV, Ravi Shankar, CN Jha, AK Remya S, Vanik D, *et al*. Coagulase gene polymorphism of *Staphylococcus aureus* in fish and its multiple drug resistant pattern. Journal of Environmental Biology. 2016;38:389-392.