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Isolation And Identification Of Pathogenic Bacteria In Edible Marine Fish, Rastrelliger Kanagurta And Scomberomorus Guttatus (Scombridae) Landing At Visakhapatnam Fishing Harbour, Andhra Pradesh

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Abstract

The study reports on the isolation and identification of certain pathogenic bacteria in commercially and nutritionally important edible marine fish, Rastrelliger kanagurta and Scomberomorus guttatus. In R. kanagurta, Escherichia coli, Staphylococcus aureus and Vibrio cholera were found while in S. guttatus, only Salmonella sp. was found. All these bacteria are pathogenic and cause sickness indicating that these fish are contaminated with microbes and need to be properly processed and cooked prior to consumption by humans. Further, it is recommended that the consumers need to be educated regarding the health issues that arise from the consumption of this fish contaminated with pathogenic bacteria.

Key words: Rastrelliger kanagurta, Scomberomorus guttatus, Escherichia coli, Staphylococcus aureus, Salmonella sp. and Vibrio cholera.

Introduction

Fish is a primary source of protein, vitamins and various minerals for both young and old human consumers in many parts of the world, especially in developing countries (Arannilewa et al. 2006; Edem 2009; Koffi-Nevry et al. 2011). Even though sea food is highly nutritive, it acts as a vehicle for the pathogenic bacteria occurring naturally in the marine environment (Wallace et al. 1999; Gillespie et al. 2001). The microbial flora in the freshly caught fish depends on the water environment from where it is harvested. The relationship that fish have with surrounding microorganisms can be mutualistic or pathogenic (Gomez and Balcazar 2008; Sullam et al. 2012). Generally, fish digestive tracts host aerobic or facultative anaerobic microorganisms and contaminate edible portions of the fish (Trust and Sparrow 1974; Bairagi et al. 2002; Saha et al. 2006). Most fish-related food borne illnesses are linked to Salmonella, Staphylococcus spp., Escherichia spp., Vibrio parahaemolyticus, Clostridium perfringens, Clostridium botulinum and Enteroviruses (Yagoub 2009).

With this backdrop, the present study was contemplated to isolate and identify pathogenic bacteria species that cause sickness or diseases in humans from two commercially important fish species, Rastrelliger kanagurta (Indian Mackerel) and Scomberomorus guttatus (Indo-Pacific Seer Fish) (Scombridae) landing at Visakhapatnam Fishing Harbour, Andhra Pradesh.

Materials and Methods

Two commercially important edible marine fishes, Rastrelliger kanagurta (Cuvier 1816) and Scomberomorus guttatus (Block & Schneider 1801) (Scombridae) (Figure 1) were collected from the landing center at Visakhapatnam Fishing Harbor, Andhra Pradesh. Two individuals for each species were placed in sterile bags and transported to the laboratory within one hour in aseptic conditions for the identification and characterization of associated pathogenic and food spoiling bacteria.

Figure 1. a. Rastrelliger kanagurta, b. Scomberomorus guttatus.

Identification of total number of bacterial colonies: The working principle of Total Plate Count (TPC) method was used for the estimation of total number bacterial colonies in the culture. Petri dishes, test tubes and pipettes were sterilized in oven at 180ºC for 2 hours. The media was sterilized in an autoclave at 121ºC for 15 minutes at 1 atmospheric pressure. After sterilization, the media temperature was maintained at 45-55°C in a water bath to keep the media from freezing. The diluent solution was prepared by dissolving phosphate buffer in 225 ml of sterile water and then sterilized in an autoclave at 121°C for 15 minutes. The sample of 25 grams was mashed first, and then dissolved into a sterile diluted solution that contained a volume reaching 10 ml to obtain dilution 10^{-1}. From this solution, 1 ml was taken and placed in a test tube containing 9 ml of a sterile diluent solution to obtain 10^{-2} dilution and continued this dilution until the dilution 10^{-5} was obtained. From each tube, 1 ml of the dilution was taken by using a pipette and put into a sterilized Petri dish. Each dilution was done in duplicate. Then each Petri dish was moved in a circle on the table so that the Plate Count Agar (PCA) media was spread evenly. As soon as PCA freezed, the Petri dish was placed in the incubator at 30°C for 48 hours.

After incubation, the total number of colony forming units (CFU) was determined. The representative colonies were sub-cultured for identification of bacteria. Bacterial numbers were calculated as the average of duplicates of each set and expressed as CFU/ gm of the homogenate. Bacteria were isolated by collecting colonies at random from agar plates. The colonies were purified by repeated sub-cultures. Microbiological analysis was carried out according to APHA (1984) using commercial media culture (Hi Media India).

Test for isolation and identification of Escherichia coli: Fish sample of 10 gm was mixed with 90 ml of buffered peptone solution. Then, 0.5 ml of sample was taken from this solution and spread on the sorbitol MacConkey Tergitol-7 agar and incubated for 24 hrs at 37°C. Isolation and identification of E. coli colonies was carried out by randomly picking up the pink colored colonies from each sample. Gram staining method was used for further identification based on cellular morphology and staining effect. One drop of normal saline was placed on the center of the slide. Then, the surface of the isolated colony in the solid media was touched by a straight wire and emulsified it in the saline drop to form a thin film which was allowed to air dry. The smear film was heat fixed while holding the slide at one end and by quickly passing the smear over the flame of the Bunsen burner 2 or 3 times. The smear on the slide was covered with crystal violet and allowed it to stand for one minute.

Later, it was rinsed gently under tap water, covered it with Gram's iodine and allowed it to stand for one minute. The smear was rinsed again gently under tap water and decolorized it with 95% alcohol. The smear was again rinsed under tap water before it was covered with safranin for one minute. After this, the smear was again rinsed under tap water and allowed it for air drying. Finally, the smear was observed under a light microscope at 10x and 100 x magnification for the morphology and to note whether it was gram-positive or gram-negative. Indole test, Methyl-red test, Voges-Proskauer test, Citrate utilization tests were also used for the confirmation of E. coli. For Indole test, the tryptophan broth taken in the tube was inoculated with isolated colony and incubated at 37°C for 24 hrs in a shaker incubator in ambient air. After incubation, a few drops of 0.5 ml of Kovac's reagent were added to the broth culture down the side of the tube and observed for color change. The presence of pink colored ring was taken as positive. For Methyl-red test, the test bacterium was inoculated to the broth medium and incubated at 37°C for 24 hrs. After incubation, 3-4 drops of methyl red reagent were added to the broth. The change in color to red was recorded as positive. For Voges-Proskauer test, the test bacterium was inoculated to the Methyl-red and Voges-Proskauer broth medium and incubated at 37°C for 24 hrs. After incubation, 2 drops of VP1 and 4 drops of VP2 were added to the broth and allowed to stand for 15 minutes. The change in color to red was recorded as positive. For Citrate test, the test bacterium was inoculated to citrate agar slant and incubated at 37°C for 24 hrs. After incubation, the change in color to blue was recorded as positive.

Test for isolation and identification of Staphylococcus aureus: Fish sample of 10 gm was mixed with 90 ml of buffered peptone water and beat in a motor pestle. Then, 0.1 ml of sample suspension was spread onto Baird Parker (BP) agar plate supplemented with egg yolk and tellurite emulsion and incubated at 37°C for 24 hrs. After incubation, the color change from yellow to grey to black was recorded as positive for S. aureus. Grams staining method as described above was used for cellular morphology and staining effect. Further, catalase test was performed to confirm the presence of S. aureus. The agar slant with the smear of the colony was flooded with several drops of 3% hydrogen peroxide. The appearance of bubbles due to production of oxygen gas was recorded as catalase-positive and the non-appearance of bubbles as catalase-negative. Coagulase tube test was also performed to record whether the broth culture is coagulase positive or negative. Three test tubes were taken to designate them as "test", "negative control" and "positive control". Each tube was filled with 1 ml of 1 in 10 diluted rabbit plasma. Then, 0.2 ml of overnight test broth culture was added to "test" labeled tube, 0.2 ml of

overnight known S. aureus broth culture to “positive control” labeled tube, and 0.2 ml of sterile broth to “negative control” labeled tube. All the three tubes were incubated at 37ºC and observed the broth suspensions at ½ hourly intervals for 4 hours. The gelling of plasma was noted as coagulase positive.

Test for isolation and identification of Salmonella: Fish sample 10 gm was homogenized with 90 ml of lactose broth, allowed to stand for 1 minute and incubated for 18-24 hrs to provide available nutrients required for the survival and repair of stressed and injured cells. About 0.1 ml of this broth was transferred to 10 ml of Selenite Cystine broth tube and another 1 ml of this broth to 10 ml of tetrathionate broth tube; both the tubes were incubated at 42°C for 24 hrs. After incubation, the broths in these tubes were shaken and a loop full from each tube was streaked onto the Petri plates of Bismuth Sulphite Agar (BSA) and Hektoen Enteric Agar (HEA). Then, they were incubated at 37°C for 24 hrs and observed for typical Salmonella colonies. Grams staining method as described above was used for cellular morphology and staining effect.

Test for isolation and identification of Vibrio cholera: Fish sample of 10 gm was homogenized with 90 ml of alkaline peptone water and stirred for 1 minute. Then it was incubated for 18-24 hrs to provide available nutrients required for the survival and repair of the cells. A loop full of this culture was taken and streaked onto the Petri plates of Thiosulphate-citrate-bile salt-sucrose agar. Then, the plates were incubated 37°C for 24 hrs and observed for the colony development. Grams staining method as described above was used for cellular morphology and staining effect.

Results

A number of aerobic and facultative anaerobic bacteria can be isolated by using Heterotrophic Plate Count. It includes both gram positive and gram negative bacteria. The TPC gives the total bacterial count present in 1 g of fish sample. They form colony forming units by counting this CFU/g. TPC mean value for Rastrelliger kanagurta was 2.76 $\times 10^5$ CFU/g and for Scomberomorus guttatus was 2.42×10^5 CFU/g. Gram-staining and biochemical tests indicated that R. kanagurta is infected with Escherichia coli, Staphylococcus aureus, Salmonella sp. and Vibrio cholera while S. guttatus is infected with only Salmonella sp. Of the four bacterial species, S. aureus is Gram-positive and facultative anaerobic while the other three species are Gram-negative of which E. coli is obligate anaerobic while Salmonella and V. cholera are facultative anaerobic.

Discussion

Contamination of seafood with bacterial pathogens at source (in the sea) primarily arises from the marine environment; when seafood is consumed in huge numbers causes illness in humans. Some species of the bacteria possibly cause gastro-enteritis in humans and these bacteria may also be present naturally in the marine or more especially in the estuarine environment. In the present study, the Gram-staining and biochemical tests indicated that R. kanagurta is infected with Escherichia coli, Staphylococcus aureus, Salmonella sp. and Vibrio cholera while S. guttatus is infected with only Salmonella sp. Clucas and Ward (1996) recorded the presence of S. aureus in natural micro-flora of fish and shell fish in U.K. In this study, S. aureus recorded in both fish species is highly pathogenic and major

causative agent of food poisoning in humans as it is known to release entero-toxins causing severe illness in gastro-intestinal tract. The presence of other isolated pathogenic bacteria can be attributed to the contamination of the process, improper handling, hygienic and sanitary conditions of Visakhapatnam Harbour. Drinking fecal contaminated water can also lead to an outbreak of the health issues due to these bacterial pathogens (Pelczar et al. 1993). E. coli is associated with enteric infections. E. coli and Salmonella spp. can survive for very long periods in tropical waters and once introduced may become adaptable to the new conditions favouring the growth of microorganisms in the environment and subsequently contaminating the fish species (Fujioka et al. 1988). According to the Recommendation of International Association of Microbiology Societies, fresh and frozen fish should be free of Vibrio (Hadin et al. 2004). In the present study, the presence of E. coli, S. aureus and V. cholera in R. Kanagurta and Salmonella sp. in S. guttatus indicates that the consumption of these species is not safe for consumption especially in regular consumers of sea food. Therefore, the study recommends that all edible fish species including the ones included in this study need to be regularly monitored for the microbial contamination and load in order to alert the consumers to take appropriate measures for the processing and cooking of the fish prior to its consumption.

References

APHA. 1984. American Public Health microbial Administration. Compendium of methods for the microbiological examination of foods, 2nd Ed. APHA. Washington DC.

Arannilewa, S.T., Salawu, S.O., Sorungbe, A.A. and Ola-Salawu, B.B., 2006. Effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (Sarotherodun galiaenus). Nutr. Health 18: 185-92.

Bairagi, A., Ghosh, K., Sen, S.K. and Ray, A.K. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. Aquaculture Inter. 10: 109-121.

Clucas, I.J. and Ward, A.R. 1996. Post-harvest fisheries development: a guide to handling, preservation, processing and quality. Chathman Maritime, Kent, UK.

Edem, D.O., 2009. Vitamin A: A review. Asian J. Clin. Nutr.1: 65-82.

Fujioka, J.T., Shaw, F.R., McFarlane, G.A., Sasaki Fujioka, T., Shaw, F.R., McFarlane, G.A., Sasaki, T. and Bracken, B.E. 1988. Description and summary of the Canadian, Japanese and U.S Joint database of stable fish tag releases and recoveries. NOAA Tech. Memo. NMFS F/NWC-13.

Gillespie, I.A., Adak, G.K., O Brien, S.J., Brett, M.M. and Bolton, F.J. 2001. General outbreaks of infectious intestinal disease associated with fish and shellfish, England and Wales, 1992- 1999. Commun. Dis. Public Health 4: 117- 123.

Gomez, G.D. and Balcazar, J.L. 2008. A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immunol. Med. Microbiol. 52: 145-154.

Hadin, EL., Radu, S., Chen, C.H. and Nishibuchi, M. 2004. Prevalence of potentially pathogenic Vibrio species in the seafood marketed in Malaysia. J. Food Prot. 67: 1469-1475.

Koffi-Nevry, R., Ouina, T.S.T., Koussemon, M. and Brou, B.K. 2011. Chemical composition and lactic microflora of Adjuevan, a traditional Ivorian fermented fish condition. Pak. J. Nutr. 10:332-337.

Pelczar, M.J., Chan, E.C.S. and Krieg, N.R., Edwards, D.D. and Pelczar, M.F. 1993. Microbiology: concepts and applications. McGraw-Hill, New York.

Saha, S., Roy, R.N., Sen, S.K. and Ray, A.K. 2006. Characterization of cellulose producing bacteria from the digestive tract of tilapia, Oreochromis mossambica (P) and grass carp, Ctenopharyngodon idella (V). Aquaculture Res. 37: 380-388.

Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M.P., Rosen, G.L. and Knight, R. 2012. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol. Ecol. 21: 3363-3378.

Trust, T.J. and Sparrow, R.A.H. 1974. The bacterial flora in the alimentary tract of freshwater salmonid fishes. Canadian. J. Microbiol. 20: 1219- 1228.

Wallace, B.J., Guzewich, J.J., Cambridge, M., Altekruse, S. and Morse, D.L. 1999. Seafood-associated disease outbreaks in New York, 1980 -1994. Am. J. Prev .Med.17: 48 54.

Yagoub, S.O. 2009. Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state. J. Bacteriol. Res. 1: 085-088.