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# OCCURRENCE OF *SALMONELLA* IN FRESHWATER FISHES: A REVIEW

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## ABSTRACT

Fishes account for approximately 17% of the global animal protein intake. Fish and fishery products, however, have been documented as a carrier of food-borne pathogens. Fishes serve as a host to a variety of parasites including *Salmonella.Salmonella*, usually, is not a fish pathogen, rather the consumption of *Salmonella* contaminated feed and water causes this infection. Smoked fishes serves as a vehicle for the transmission of *Salmonella* that exists on skin, in gills and intestine. The absence of a suitable hygieneprogramme to overcome the transmission of *Salmonella* in the fisheries production sector may be the main reason for the spread of the diseases. The impact of this pathogen in human, particularly in the very young or older, includes gastroenteritis, abdominal cramps, enteric fever and bacteremia resulting from ingestion of uncooked fishes. In spite of these unhealthy impacts, their epidemiology is still poorly understood. Studies are, therefore, needed to summarize the impact of *Salmonella* infected fishes, with particular attention to freshwater fishes in human.

**Keywords:**Human pathogen, *Salmonella*, contaminated feed, freshwater fishes.

## INTRODUCTION

Fishes accounts for approximately 17% of the global animal protein intake (FAO, 2014). Globally, the production of fish compete the growth of world population (FAO, 2014). In a world where more than 70% of the planet is covered with water, aquatic foods may provide an essential component of the global food to improve nutrition, health, and well-being of humans(Tacon and Metian, 2013). In developing countries, despite the low consumption of fishes by weight, it contributes 180 kilocalories per capita per day, in a few countries with a developed fish preference(Lokuruka, 2009). Fish is a source of unsaturated fats, called omega-3 fatty acid, which affect cardiac functions includinghemodynamics and arterial endothelial function (Wolfe, 2010). Fish is an excellent source of high quality protein, contains the essential amino acids that are necessary for human health (Hoyle and Merritt, 1994). Fishes skin surface, intestine and gills, however, carries high microbial load ($1.72 \pm 0.68 \times 108$ to $7.00 \pm 3.39 \times 108$) (Mhango *et al.*, 2010). Food safety hazards in aquaculture include contaminants including environmental pollutants, fish disease, and hygienic aspects (microbial agents) (Hastein *et al.*, 2006). The hazard factors during handling of fishes may include catching, slaughtering and processing for consumption(Hastein *et al.*, 2006). The natural habitat of fish is extremely susceptible to pollution from domestic, industrial and agricultural discharges. Therefore, fish and other aquatic life forms are vulnerable to all environmental hazards (Raufu *et al.*, 2014).

The common human pathogenic diseases that transmitted from fishes caused by patients' contact with fish, aquatic environment, dietary habits, and immune system status of the exposed individuals are fairly common (Akoachere *et al.*, 2009). The pathogenic diseases are usually caused by bacterial species which are facultative pathogenic for both fish and human. The microbiological safety of aquaculture products has been a source of concern to consumers, industries, and regulatory agencies all over the world (Novotny *et al.*, 2004). The quick and exact identification of bacterial pathogens is equally important for quality declaration and to trace bacterial pathogens in the food supply (Germini *et al.*, 2009). Infection as a result of microbial contamination does not usually result in a disease but environmental stresses may upset the balance between the potential pathogens and their hosts (Iqbal *et al*., 2012).

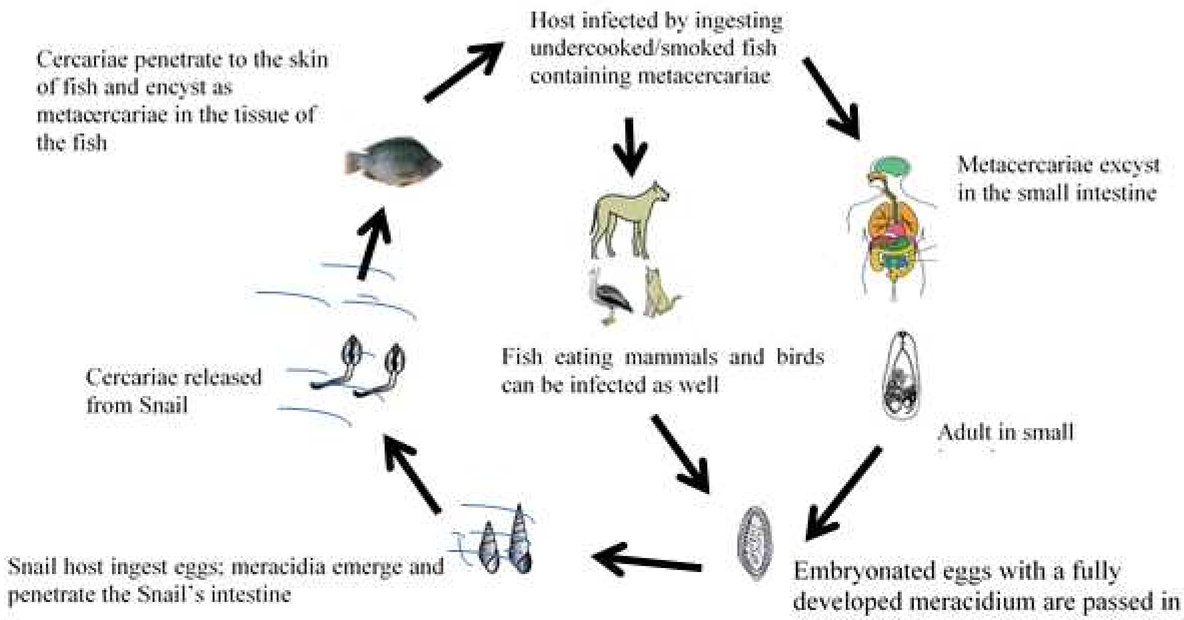


Figure 1. Life cycle and transmission of *Salmonella.*

*Salmonellosis* occurrence in fishes is thought to depend on the interaction of three variables (Olgunoğlu, 2012), which are controlled by abiotic, biotic, and genetic factors (Rozen and Belkin, 2001). These variables include quality of the environment, and differential susceptibility of individuals to the pathogen as a result of genetic inclination (Rozen and Belkin, 2001). The presence and virulence of the pathogen can also be included in these factors (Kaur and Jain, 2012).

*Salmonella* is a gram negative, rod shaped, pathogenic bacteria of water bodies. These bacteria pose a great threat on human health in warm climatic zones (Heinitz *et al.*, 2000). *Salmonella enteritidis* is the main culprit in fishes. Other *Salmonella* spp. including *typhimurium*, *agora*, *Montevideo* and*enteretia* are also clinically significant. Reported cases of *Salmonella* show a distinct consistent seasonal pattern, with a peak of infection observed during August. The wide range of human diseases that caused by *Salmonella*includes, enteric fever, bacteremia and gastroenteritis. Gastroenteritis has the utmost adversative effect on children's growth and improvement (Nwiyi and Onyeabor, 2012). *Salmonella* is a second leading cause of foodborne illness worldwide (Wong and Chen, 2013). The majority of 1.3billion annual cases of *Salmonella* cause human gastroenteritis, through the ingestion of undercooked eggs, shell fish and fish (Awuor *et al.*, 2011). The major reservoirs of the *Salmonella* spp. are aquatic environment; however, fish and fishery products have been renowned as a carrier of food-borne pathogens (Upadhyay *et al.*, 2010). Numerous commensal and pathogenic bacteria, which are related to fish, can grow in a low temperature; therefore, some exemptions are there. The objective of the study is to determine the occurrence of *Salmonella* spp. in freshwater fishes and tounderstand how contaminated food and contaminated environment influence the health.

***Salmonella*species:** *Salmonella* belongs to *Enterobacteriaceae* family. They are common water-borne bacterium which may be apparently present in the tissues of normal fishes(Newaj-Fyzul *et al.*, 2008). The injury or environmental stress in fishes causes serious outbreaks of the diseases with mortality (Thillai Sekar *et al.*, 2008). The environmental stresses such as high temperature and poor water quality mainly contribute to the start and severest of *Enterobacteriaceae* infections in fishes (Zheng *et al.*, 2004).Members of the genus *Salmonella* are ubiquitous, found in all organisms including humans (Lotfy *et al.*, 2011). *Salmonella* survival in water depends on biological (macro and micro-invertebrate) and physical factors (e.g. temperature). There are two species, *S. bongori*and *S. enteric* (de Freitas Neto *et al*., 2010) and around seven subspecies and various serovars of *Salmonella* (Porwollik *et al.*, 2004). More than 2500 different serotypes of subspecies (Arrach *et al.*, 2008), furthermore, are closely related microorganisms, and can infect people. First the *Salmonella* was isolated from a pig, suffering from hog cholera, by an American scientist Dr. D. E. Salmon in 1985. *Salmonella* spp.may be present naturally in tropical aquatic environments (Musefiu *et al.*, 2011). It is well known that aquatic birds spread*Salmonella*and other pathogens in the environment that are associated in fish-borne diseases of humans. During handling of fishes, the natural flora of aquatic environment will be contaminated with organisms associated with human, such as *S. typhii*and*S. aureu,*both isolated in this investigation, can grow well at 30 to 37 $^{0}$C (Ibrahim and Sheshi, 2014).

***Salmonella*from fishes:***Salmonella* in freshwater fishes has been usually related to the fecal contamination of water from where fish were harvested (Mhango *et al.*, 2010). Fishes work as a passive carrier of *Salmonella* that may excrete *Salmonella* spp.without apparent symptoms and represent no clinical disease. The high prevalence of *Salmonella* in catfish was reported by Wyatt *et al.* (1979b). This high prevalence rate was attributed to the high temperature in pond water because high temperature promotes the growth rate of the organism (Wyatt *et al.*, 1979a). *Salmonella* was isolated from the intestines of Silver Carp by Bocek *et al.* (1992). Silver Carp has thus shown to be potential carriers of *Salmonella*. The microbial contamination of fishes grown in ponds in and around Calcutta was reported by Pal and Gupta (1992). The later authors observed a significant linear correlation between *Salmonella* of the pond water and their recovery from the tissues of the fishes.

The presence of different serovars of potentially human pathogenic *Salmonella* among largemouth bass, channel catfish, common carp and sucker mouth catfish was isolated from the fishes of natural river system. The fish gut samples were collected and analyzed for *Salmonella*. For this purpose, the combination of molecular detection and identification tools with traditional enrichment culture technique, such as polymerase chain reaction (PCR) and in situ hybridization, were used(Gaertner *et al.*, 2008). The contents of blood, tissues, intestine and skin surface were compared in a freshwater dam fish. *Salmonella* spp. were present in all parts of the fishes(Cahill, 1990). The bacterial load was studied through pour plate method from fresh and smoked fishes(Ibrahim and Sheshi, 2014). The prevalence of *Salmonella* in different body parts of fishes was studied by Mohamed Hatha and Lakshmanaperumalsamy in 1997. The prevalence of *Salmonella* isolated from freshwater lake, farmed and market fishes were 31%, 5% and 10 to 28%, respectively (Mohamed Hatha and Lakshmanaperumalsamy, 1997). The pathogenic *Salmonella* was isolated from smoked fishes by Mailoa and St Sabahannur (2013) that can cause food poisoning. The latter results showed a relatively high level of incidence of *Salmonella* in the intestine, skin and gills of fishes, respectively than in mouth, liver and muscles (Tables 1 and 2).

**Table 1.Occurrence of *Salmonella* in different organs of fish.**

| Species | Infected organ | | | | | | References |
|---|---|---|---|---|---|---|---|
| | Skin | Mouth | Intestine | Liver | Gills | Muscle | |
| Tilapia (*Oreochromisnilonica)* | × | × | × | ✓ | ✓ | ✓ | Lotfy *et al.*, 2011 |
| Tilapia (*Tilapia nilotica*) | ✓ | × | ✓ | × | ✓ | × | Nwiyi and Onyeabor, 2012; Elhadi, 2014 |
| Catfish | ✓ | × | ✓ | ✓ | ✓ | ✓ | Wyatt *et al.*, 1979b; Lotfy *et al.*, 2011; Elhadi, 2014 |
| Common carp (*Cyprinuscarpio*), Channel catfish (*Ictaluruspunctatus*), Lagermouth bass (*Micropterussalmoides*), Suckermouth catfish (*Hypostomusplecostomus*) | × | × | ✓ | × | × | × | Gaertner *et al.*, 2008 |
| Tilapia (*Tilapia rendali*), *Oreochomismossambicus* | ✓ | ✓ | ✓ | × | ✓ | × | Sichewo *et al.*, 2013 |
| Thaila (*Catlacatla*), Silver carp (*Hypophthalmichthysmolitrix),* *Ctenophryngodonidella* | ✓ | × | × | × | × | × | Balasubramanian *et al.*, 2012 |
| Rohu (*Labeorohita)* | ✓ | × | ✓ | × | ✓ | × | Balasubramanian *et al.*, 2012; Elhadi, 2014 |
| Goldfish (*Carassius auratus),* Grass carp (*Ctenopharyngodonidella)* | ✓ | × | ✓ | × | ✓ | × | Cahill, 1990 |
| Carfu, Mirgal, Milkfish, Mackerel | ✓ | × | ✓ | × | ✓ | × | Elhadi, 2014 |

**Table 2.Prevalence (%) of *Salmonella* spp. documented in literature.**

| Species | Intestine (%) | Gills (%) | Overall body (%) | References |
|---|---|---|---|---|
| Fish | 41.3 | - | 10.4 | Rahimi*et al*., 2013 |
| Scopelidae | - | - | 28.0 | Mohamed Hatha and Lakshmanaperumalsamy, 1997 |
| Trachnidae | - | - | 26.9 | Mohamed Hatha and Lakshmanaperumalsamy, 1997 |
| *Tilapia nilotica* | 20.0 | 50.0 | 66.7 | Nwiyi and Onyeabor, 2012; Elhadi, 2014 |
| Catfish | 20.0 | 40.0 | 60.0 | Budiati*et al.*, 2011; Elhadi, 2014 |
| Carfu | - | - | 27.7 | Elhadi, 2014 |
| Mirgal | - | - | 35.0 | Elhadi, 2014 |
| Milk fish | - | - | 52.0 | Elhadi, 2014 |
| Mackerel | - | - | 31.4 | Elhadi, 2014 |
| Rohu | - | - | 46.6 | Elhadi, 2014 |

**Clinical signs, pathology and pathogenesis:**Clinical signs related with *Salmonella,* infected fishesmay include septicemia and bacteremia, whereas fish in the absence of clinical signs, can shed *Salmonella*(Bocek *et al.*, 1992). Fish feed is considered as a major source of *Salmonella* infection in commercial fish farms. Generally, with the exception of *Salmonella Arizona*, the *Salmonella* have not been considered as a fish pathogen (Austin and McIntosh, 1991). The *Salmonella* spp. is carried through fish farmed, fish meal, and fresh fish. When the fishes are caught incontaminated areas with fecal pollution, processed, and distributed under unsanitary conditions and slightly cooked (Norhana *et al.*, 2010). The maximum prevalence of *Salmonella* in fishes indicated the unhygienic handling and transportation practices from fish resources to market. Environmental conditions during the monsoon season, may also favor a high degree of secondary adulteration, as well as prolonged survival of these organisms in their natural ecosystems (Mohamed Hatha and Lakshmanaperumalsamy, 1997). The presence of *Salmonella* spp. in live catfish skin and internal organs facilitate cross contamination to the dead organisms through processing procedures (Heinitz *et al.*, 2000). Fish that is a passive carrier of *Salmonella* represent no clinical disease and may excrete *Salmonella* spp. without external trouble.

The *Salmonella* infection in human being and other animals occur due to the ingestion/consumption of under cooked fish contaminated during processing (Novotny *et al.*, 2004). The factor that may have contributed to the contamination of fishes through *Salmonella* includes unconventional use of poultry and cattle feces as a fertilizer on fish farms. Resultantly, during the rainy season the top soil layer is washed in to the fish pond/river leading to food contamination. These have enormously contributed to increasing population of human-fish microbes. The poor sewage disposal couples with a high water table permits untreated sewage to enter fish farms through runoff. The other factors includes packaging of fish in contaminated container, fishing boats, with the methods of displaying uncovered in open market and transportation. Washing of the fish with lake water may also exacerbate the contamination process. Therefore, there is a dire need to investigate the source of *Salmonella* spp. infecting humans (Raufu *et al.*, 2014).

*Salmonella enteric* can cause asymptomatic carrier state, gastroenteritis, bacteremia and enteric fever (Ryan and Ray, 2010; AbMutalib*et al*., 2014). It is more common in children under the age of 5, and patients of 70 or above. Gastroenteritis is commonly identified by unexpected nausea, abdominal cramps, diarrhea, continuous pain in head, cold and fever up to 39ºC (Newton and Surawicz, 2011). The symptoms of salmonellosis can be insignificant to severe and may last between 5 to 7 days(Ryan and Ray, 2010).Bacteremia occurs in 3 to 10% of individuals infected with*Salmonella enteric* have higher mortality rate (Bronze and Greenfield, 2005). Enteric fever or typhoid fever is caused by serotypes of *Salmonella*, which includes *Typhi* and *Paratyphi*(Lotfy *et al.*, 2011). The detected rate of *Salmonella* in fish meal was 8%. *Salmonella* in naturally contaminated feeds ranged from <3 to 21 *Salmonella*/100 g (Starkey, 2013).

**Epidemiology of the disease:***Salmonella* is the causative agent of salmonellosis, a severe form of human gastroenteritis (Bangtrakulnonth *et al.*, 2004). In humans, *Salmonella* infection may range from a self-limited gastro-enteritis, typically associated with non-typhoidal*Salmonella* to typhoidal fever with lethal intestinal perforation. The annually, worldwide predictable food poisoning incidence are 1.3 billion cases, whereas 3.0 million deaths per year. *Salmonella enterica*infections occur worldwide. Their symptom includes vomiting, abdominal cramps, fever, head-ach, chill, enterocolitis, diarrhea, blood in the stool and muscle-ach. Such types of symptoms usually appear between 12 to 72 hrs afterward the bacteria have been ingested and last anywhere from 4 to 7 days. The individual with good health can usually get rid themselves. The children, elder people and the individuals with weak immune systems may need treatment (Coburn *et al.*, 2006).In industrialized countries, the non-typhoid salmonellosis is more common, whereas in developing countries enteric fever mostly found. *Salmonella* spp. reached to aquatic ecosystem through fecal contamination and in many countries, has been isolated from freshwater fish culture ponds (Lotfy *et al.*, 2011). Human infection usually occurs when consuming contaminated foods and water, contact with infected feces, as well as contact with infected animals. Humans can transfer the disease as long as they shed the bacterium in their feces.

**Diagnosis:** *Salmonella* spp. (enteric bacteria) may present in fish and fishery products due to fecal contamination and bacterial contamination during storage, processing, and preparation for consumption. In dense populations of cultured food, aquarium fishes or fish farm the epidemics of bacterial diseases are common. Susceptibility to such outbreaks commonly is associated with organic loading of the aquatic environment and poor water quality (Francis-Floyd, 2011). The other factors may include handling, processing and transport of fishes, hypoxia, sudden changes in temperature, or other stressful conditions (Olsvik *et al.*, 2013). Most bacterial pathogens of fishes can be diagnosed by isolation of the organism in pure culture from infected tissues and identification of the bacterial agent (Toranzo *et al.*, 2005). XU *et al.* (2007)and Law *et al.* (2015) used multiplex PCR to detect food borne bacterial pathogens. The occurrence of *Salmonella* spp. in aquatic food was investigated by standard microbiological techniques and polymerase chain reaction (Zadernowska and Chajęcka, 2012). The presence of salmonellosis may be confirmed by the isolation of organisms from feces or blood (Salmonellosis, 2005).

The factor that may have contributed to the contamination of fishes with *Salmonella*, originated from terrestrial sources such as untraditional utilization of cattle and poultry feces as fertilizer or manure on farmland located close to the canal, river or pond. Consequently, during the rainy season the top soil washed into the water reservoir enhance the fish and environmental contamination. These have mainly contributed to the growth of human and fish microbes. The untreated sewage water when enter to the lakes or fish farm through runoff or storm water contaminated the fishes. The transportation of fishes in contaminated fishing boat, or containers and washing of fish with pond/lakes water may also lead to aquatic environment and food contamination (Raufu *et al.*, 2014). There is a need to established conventional methods to detect and identify pathogens and implement preventive and control measured.

**Treatment:**The treatment can be categorized in to two approaches, (a) physical and (b) chemical approaches. The physical approach may include proper cooking, refrigeration, irradiation, and modified atmosphere packaging (Olgunoğlu, 2012). In cooking, the application of heat at 99-100°C for 3 to 4 minutes is acceptable for safe processing of fishes before consumption. This temperature is sufficient to kill vegetative forms of pathogens (Olgunoglu, 2010). The *Salmonella* unstressed cells are heat sensitive and easily damaged at pasteurization temperature (Sharan *et al.*, 2010). The maximum cumulative exposure time at 5.2 to 10°C is for two days, at 11-21°C for 5 hours and at >21°C for two hours to control the growth of *Salmonella* in fish and fishery products. The well-known methods to extend the shelf life of food items are freezing and refrigeration. These procedures stopped the metabolic processes of bacteria, also decrease chemical and biochemical reactions (Norhana *et al.*, 2010). The minimum temperature reported for the growth of *Salmonella* spp. (*S. typhimurium*) is 6.2°C (Ingham *et al.*, 1990). The growth of *Salmonella* may be prevented through proper refrigeration (Ingham *et al.*, 1990). Therefore, proper sanitation after refrigeration of aquatic food items such as proper cooking is very important in maintenance of the quality. For the long term preservation, safety and improvement of quality of the fishery products can be used irradiation method. For this purpose, fishery products involved the direct exposure to electromagnetic rays (Oraei *et al.*, 2011). The irradiation dose reported for *Salmonella enteritidis*is 3.0 killo gray, which effectively control the pathogen. Similarly to attain safety levels beside *Salmonella* spp., particularly *S. enteritidis*, in raw oysters, a dose of 3.0 killo gray is recommended. To extend the shelf life of fish and fishery products modified atmosphere packaging has been widely used since 1980. The efficiency of this method in eliminating pathogens from fishes depends on the gas mixture, which includes $CO_2$, Nitrogen, 0.4 percent of carbon monoxide and storage temperature (Hudecová *et al.*, 2010). In fresh chilled common carp (*Cyprinuscarpio*), no *Salmonella* growth was observed during storage at +4 ± 0.5 °C for 10 days in two different modified atmosphere packaging (70% N2/30% CO2; 80% O2/20% CO2) (Olgunoğlu, 2012). In modified atmosphere storage including proper refrigeration, significant decrease in growth rate of *S. typhimurium* was observed at dose rate of 50% CO2/10% O2 (Ingham *et al.*, 1990).The chemical approach includes the use of antimicrobial agents was used by Olgunoğlu, (2012). Studies indicated that lactates stimulates bacterial metabolism, for instance intercellular acidification and feedback inhibition (Da Silva 2002). Lactate is considered to be an effective additional hurdle against the growth of contamination flora and pathogens such as *Salmonella* and it is used in the further processed fish industry (fish cakes, smoked salmon, injected fillets, marinated fish) (Olgunoğlu, 2012).

**Conclusion:** Isolation of*Salmonella*from fishes is an indication of contamination of the river/lakes by pathogens. This study provides vital data that are critical for assessing and controlling the risk associated with the presence of*Salmonella*e in fishes. It is obvious from the literature reviewed here that consumption of*Salmonella*infected fishes can increase public health problems. The contamination of fishes through aquatic environment, contaminated by humans and poultry itself, may create a secondary food reservoir in endemic areas. There is a need to educate the public on the danger associated with consumption of raw or improperly cooked fishes. Quality control measures should be introduced for export oriented and locally consumed fish. It is suggested, therefore, that fishes should be properly processed before consumption. There is a need for regular surveillance of fishes sold for consumption to ascertain the level and types of antimicrobial resistance. This will enable prompt and effective control measures.*Salmonella*surveillance can provide data to formulate control measures for effective treatment and prevention of food born and zoonotic pathogens.

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