Salmonella spp. in the fish production chain: areview

Salmonella spp. na cadeia produtiva do peixe: uma revisão

Dandara Virginia Guia Semedo Fernandes[1], Vinicius Silva Castro[1,2], Adelino da Cunha Neto[3], Eduardo Eustáquio de Souza Figueiredo[1,3,4*]

ABSTRACT

Salmonella spp is a pathogen responsible for severe foodborne infections, can be introduced into the fish production chain through inadequate handling or hygiene or contact with contaminated water, and is not a biological contaminant originally reported in fish. Fish microbiological safety is a concern for consumers, industries and regulatory agencies worldwide, since fish, an important food category in the international trade and often exported to several countries, can act as a vehicle for *Salmonella* transmission throughout the production chain.In addition, concerns regarding the misuse of antibiotics in aquaculture are also an issue, as a result of the increased isolation of resistant and multiresistant *Salmonella* serovars. In this review, we examined aspects associated with the microbiological risks of the presence of *Salmonella* spp. in fish and their implication in the aquaculture production chain. In addition, incidence and antimicrobial resistance data are presented, as well as strategies for *Salmonella* prevention and control in fish.

Key-words: aquaculture, enterobacteriacae, antibiotic, antimicrobial resistance, salmonellosis.

RESUMO

Salmonella spp.é um patógeno responsável por uma grave infecção alimentar que pode ser introduzida na cadeia do pescado, por meio da manipulação e higiene inadequada ou por contato do peixe com águas contaminadas. Essa bactéria não é contaminante natural no

[1]Programa de Pós-graduação em Ciência Animal, Universidade Federal de Mato Grosso (UFMT), Cuiabá, MT, Brasil.
[2]Programa de Pós-graduação em Ciência de Alimentos, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ, Brasil
[3]Faculdade de Nutrição, UFMT, Cuiabá, MT, Brasil.
[4]Programa de Pós-graduação em Nutrição, Alimentos e Metabolismo, UFMT, Cuiabá, MT, Brasil. E-mail: figueiredoeduardo@ufmt.br. *Corresponding author.

pescado. Por isso, a segurança microbiológica do pescado é uma preocupação dos consumidores, indústrias e das agências reguladoras em todo mundo, uma vez que o pescado é importante produto para comércio internacional de alimentos, sendo frequentemente exportados para vários países podendo ser veículador da transmissão de *Salmonella* em toda a cadeia produtiva. Outro aspecto preocupante é quanto ao uso inadequado de antibióticos na aquicultura, o que vem resultando no aumento do isolamento de sorovares de *Salmonella* resistentes e multidroga resistentes. Nesta revisão, são examinados aspectos associados ao risco microbiológico da presença de *Salmonella* spp. no pescado e sua implicação na cadeia produtiva da aquicultura. Dados sobre a incidência e resistência antimicrobiana e estratégias de prevenção e controle de *Salmonella* no pescado no Brasil e no mundo são apresentados e discutidos.

Palavras-chave: aquacultura, enterobacteriacae, antibióticos, resistência antimicrobiana, salmonelose.

INTRODUCTION

Fish are seen as a substitute for beef, pork and poultry, since consumers now demand meat containing less fat, to meet a modern and healthy lifestyle (CBI, 2016). Thus, the agricultural outlook of the UN Food and Agriculture Organization (FAO) for fish production is the production of 195 million tonnes by 2025, while consumption is expected to reach 21.8 kg per capita per year, on all continents.

To meet the growing demands for fish, safety is an important factor to consider, since these animals may be vehicles for the transmission of certain pathogens, such as *Salmonella*. In fact, the European Food Safety Authority reported that food products that most caused foodborne in Europe in 2016 were chicken (9%), cheese (8%) and fish (7%). *Salmonella* was responsible for 7.5% of cases associated with outbreaks in Brazil in 2016, while this bacterium caused 7,728 cases of foodborne in 2015 in the United States, representing a

15.89% incidence (CDC, 2016). In Europe, a total of 4,362 food-borne outbreaks were reported, most of them caused by bacterial agents, in particular *Salmonella* spp., which accounted for 21.8% of all outbreaks in 2015 (EFSA, 2016).

Salmonella is not a biological contaminant originally reported in fish, being introduced through contaminated water or improper handling. The fact that this bacterium survives in soil and water and may be transfered to fish plays an important role in providing information on the nature of the contamination and the possible dissemination route of this bacterium, thus allowing traceability of the microbial source in fish slaughterhouses.

In this context, a literature review was carried out on *Salmonella* in fish and its implications in the production chain of aquaculture fish, in order to better understand the contamination dynamics with the aim of proposing preventive actions.

Salmonella

Salmonella belongs to the *Enterobacteriaceae* family, characterized as a non-sporulated gram-negative bacillus. It is routinely classified by serotype, based on the expression of three types of antigens: (O) somatic, (H) flagellar and (vi) capsular, according to the Kauffmann-White scheme (BRASIL, 2011). This current classification scheme is based on two main *Salmonella* species: *S. enterica* and *S. bongori*. In this scheme, *S. enterica* is further classified into 6 sub-species: *S.enterica*subspecie *enterica* (I); *S.enterica*subspecie*salamae* (II); *S. enterica*subspecie*arizonae* (IIIa); *S. enterica*subspecie*diarizonae* (IIIb); *S. enterica*subspecie*houtenae* (IV) and *S. enterica*subspecie *indica* (V). Currently more than 2,600 serovars have been identified, including 1,547 belonging to *S. enterica enterica* (DEKKER &FRANK, 2015), which causes 99% of diseases in humans and animals (GRIMONT &WEILL, 2007; CDC, 2008; ISSENHUTH-JEANJEAN et al., 2014).

The *S.* enterica subsp. enterica serovar Typhimurium and Enteritidis are the most common and can cause diseases in several animals (THOMSON et al., 2008; EFSA/ECDPC, 2017). Some serogroups are adapted to specific species. For example, *S. enterica* Typhi and *S. enterica* Paratyphi are restricted to humans, causing typhoid and paratyphoid fever, respectively (HOLT et al., 2009). Those adapted to cattle, *S. enterica* Dublin, and to pigs, *S. enterica* Choleraesuis, are occasionally reported in humans, causing severe ailments (SIRICHOTE et al., 2010; GAL-MOR, BOYLE & GRASSL, 2014).

Salmonella is a facultative anaerobic, and oxidase-negative, usually mobile, that produces gas from glucose. Its growth temperature ranges from 7° C to 46° C, with temperature optimum ranging from 35° C to 43° C and, growth pH ranging from 3.8 to 9.5, with optimum pH between 7.0 and 7.5. Minimum water activity for growth is 0.84 and optimum salt concentration is up to 4% (JARVIS et al., 2016). Regarding biochemical identification, *Salmonella* is catalase and methyl red positive, and indole, vogues proskauer, malonate and urea negative. It produces hydrogen sulphide gas (H_2S) from the reduction of sulfur through cysteine desulphydrase and displays as metabolic characteristics decarboxylation capacity regarding the amino acids lysine and ornithine, nitrate to nitrite reduction and the use of citrate as the only carbon of (BRASIL, 2011).

Pathogenicity mechanisms

In humans, salmonellosis causes gastroenteritis, bacteremia and more serious systemic diseases, such as typhoid and typhoid fever (BIBI et al., 2015). Infection mechanism by *Salmonella* spp. in humans institutes several steps along the gastrointestinal tract for the infection to be able to occur in the host. However, the bacteria must invade multiple gut defenses until gaining access to the epithelium. *Salmonella* spp. presents an adaptive tolerance response to acidic environments, such as the stomach, that allow for its survival under these conditions. When arriving in the intestinal mucosa, it adheres to the epithelium by means of fimbriae, which facilitate attachment to the intestinal epithelium (Figure1) (MADIGAN et al., 2010; MONACK, 2012; THOMPSON et al., 2017).

The M cells and enterocytes are the main access ports to the pathogen. From M cells, a transcytosis process occurs, and *Salmonella* reaches the submucosa, where it comes into contact with phagocytes and activates virulence mechanisms, allowing its survival and replication in phagocytes (OLIVEIRA et al., 2013; WOTZKA et al., 2017).

SopE, SopE2 and *SopB* proteins act together to trigger changes in the cytoskeleton of host actin cells. Since *Salmonella* crosses the epithelial lining, secretion of type III protein (T3SS-2) allows the pathogen to survive in mononuclear tissues, such as macrophages and dendritic cells (THIENNIMITR et al., 2012; WOTZKA et al., 2017).

Endocrine *Salmonella* then resides and proliferates within a modified vacuole. These vacuoles perform transcytosis to the basolateral membrane. Simultaneously, the induction of a secretory response in the intestinal epithelium leads to phagocyte recruitment and transmigration from the submucosa to the intestinal lumen (Figure1).

Salmonella in aquaculture

Several factors influence the risk of microbiological contamination in aquaculture products, mainly location, cultivated species, breeding practices, processing and cultural habits. Some of these potential microbiological hazards may be associated with poor hygiene patterns and sewage and livestock drainage. Leaching, for example, carries environmental pollutants into river waters and; consequently, fish, causing deleterious effects in this system (SETTI et al., 2009; TRAORÉ et al., 2015).

Another factor contributing to *Salmonella* contamination in fish is the use of poultry litter as fertilizer in culture tanks (ESPOSTO et al., 2007; AMPOFO & CLERK, 2010), as well as rearing in consortium with other types of animals, such as poultry, cattle and pigs (LI et al., 2017), which contributes to the increase of the microbial population in fish tanks.Consequently, fish may undergo physiological changes due to direct access of contaminants to the bloodstream through gills (MOUNTAIN et al., 2011; GOSSNER et al., 2015).

Fish stress factors, such as incorrect management practices, unbalanced diets or tank overcrowding, can increase susceptibility to diseases and facilitate the spread of pathogens by decreasing immune responses, thus altering fish efficiency in combating infectious agents (AMAGLIANI &SCHIAVANO, 2012). Once contaminated, fish may become *Salmonella* hosts and present no clinical manifestations (BIBI et al., 2015). Some studies have demonstrated the high incidence of *Salmonella* in fish intestines, skin and gills (NWIYI & ONYEABOR, 2012), as well as muscle (EL-OLEMY et al., 2014). *Salmonella* presence on fish surface and internal organs facilitates cross-contamination during fish processing (HEINITZ et al., 2000).

Cross contamination and prevalence in fish

Salmonella is not a normal bacterial component of fish microbial flora, and the occurrence of this pathogen is commonly related to its breeding , as well as to the industrialization environment, due to inefficient hygiene practices, equipment and inadequate food handling. Therefore, fish slaughterhouses must take special care in the handling, storage, preservation, transport and commercialization of these animals, since the quality of the final product depends on these factors.

Fish contamination in slaughterhouses can occur during all processing stages, such as transportation, washing with hyperchlorinated water, evisceration, peeling, and filleting, as well as contact with ice used for conservation, contaminated water, boards, knives, trays and plastic boxes.

Thus, adequate sanitary conditions during processing, including handler hygiene, as well as that of the surfaces used for fish manipulation, such as tables and utensils, in addition to the use of clean and chlorinated water during all processing steps, are essential to avoid cross contamination (DUARTE et al., 2010).

One of the most important factors regarding *Salmonella* cross-contamination is the fact that this bacterium can remain viable on food contact surfaces for significant periods. This is due to the formation of biofilms, a well-known bacterial mode of survival that protects bacteria from stressful environmental conditions, such as drying and cleaning procedures (CARRASCO et al., 2012). Cross-contamination associated with raw and processed food through contact surfaces is considered a potentially dangerous event, and some authors have reported the presence of *Salmonella* on stainless steel surfaces (WANG et al., 2015).

Contamination also occurs due to the inadequate sanitary status of food handlers who have salmonellosis and who become carriers during a certain period after the disease symptoms have subsided. These carriers excrete *Salmonella* bacteria in faeces and, because of insufficient personal hygiene, create public health risks, since they may cause fish contamination when handling food during the unloading, processing or preparation steps.

Control strategies to prevent fish-associated diseases include the identification and implementation of hygienic-sanitary controls during processing (AMAGLIANI &SCHIAVANO, 2012). In the industrial environment, the most commonly applied program to guarantee food quality is the HACCP-based system (Hazard Analysis and Critical Control Points), which establishes control measures at pre-established points in the processing line and CP (control point) and/or CCP (critical control point) monitoring, as well as management practices focused on record-keeping and corrective actions (CORNIER et al., 2007).

The incidence of salmonellosis due to fish consumption has become a concern for public health agencies in several countries, due to the significant increase in consumption of aquaculture produces, especially raw products, which increase pathogen exposure risks, especially in vulnerable groups, such as elderly, pregnant women and infants (ZHANG et al., 2015; PAUDYAL et al., 2017).

The importance of this pathogen in fish can be assessed and evaluated, since records indicated that fish were responsible for the occurrence of 7% of total foodborne outbreaks in Europe in 2016, with the majority of *Salmonella* infections in humans related to fish consumption caused by the Typhimurium and Enteritidis serovars (SANTIAGO et al., 2013; EFSA, 2016).

In the United States, this bacterium was responsible for 20,000 admissions and 380 deaths per year over a three-year period (BAE et al., 2015). In 2015, also in the US, a *Salmonella* outbreak was identified, with the recall of frozen raw tuna (*Thunnusalalunga*) from an Indonesian industry. This led to 65 people becoming infected with *Salmonella* Paratyphi B and *Salmonella* Weltevreden in 11 American states (CDC, 2015).

Another study, conducted on 11,3120 seafood samples imported from different countries over a 9-year period in which they were collected by the Food and Drug Administration (FDA) concluded that the overall incidence of *Salmonella* spp. in seafood was of 7.2%, and that incidence rates are higher in Central Pacific and African countries, or developing countries, compared to developed countries, such as countries in Europe, including Russia, and North America, that displayed a significantly lower incidence. (HEINITZ et al., 2000).

In general, the Typhimurium and Enteritidis serotypes are the most prevalent in developed countries; although, other serotypes predominate in specific regions, such as the

Stanley and Weltevreden serotypes in Asia (Table 1). These differences can be justified due to differences in the way animals are raised and the large trade flow between different countries.

Studies have pointed to a 10.4% (19/384) prevalence of *Salmonella* in fresh fish in Iran, where five different serotypes were detected, namely *S.* Typhimirium, *S.* Enteretidis, *S.* Typhi, *S.* Paratyphi B and *S.* Newport.

In Brazil, few studies on *Salmonella* in fish are available. However, in the northeastern region of the country, a 5% occurrence of this bacterium in fish and captive crustaceans has been reported (DUARTE et al., 2010), as well as 18.5% in salted and dried shrimp and fish meal, respectively, marketed in retail markets of the city of Belém, in the state of Pará (SILVA & FRANCO, 2013), and 3.4% contamination incidence in Nile tilapia (*Oreochromis niloticus*) was also reported in the same state.

Microbiological standards for fish processing and marketing

Each fish-importing country establishes its own microbiological and physico-chemical standards, while each importing company also has its evaluation criteria, usually of a confidential nature. In Brazil, before being commercialized, the fish are inspected by the Ministry of Agriculture, Livestock and Supply (MAPA). When leaving the industry, the inspection responsibility passes to the Ministry of Health and in the different states, the responsibility is transferred to the respective state health departments through the sanitary surveillance system. All food control and inspection involve its own legislation, laws, decrees, resolutions, ordinances and technical standards (FARIAS & FREITAS, 2008).

In Brazil, Resolution RDC No. 12 of January 2, 2001 by the National Agency of Sanitary Surveillance - ANVISA (BRASIL, 2001), defines the microbiological criteria for foods exposed for sale and export. In item 7 of Annex I, the resolution addresses the maximum levels for microorganisms in "chilledor frozen fish, not consumed raw". The criterion of absence of *Salmonella* in foods complies with World Health Organization (WHO) recommendations.

Fish are passive *Salmonella* transporters that can excrete the bacteria without apparent symptoms or clinical manifestations. In these cases, when there is no efficient sanitary barrier, such as washing with hyperchlorinated water and quality control programs (GMP, HACCP, PPHO), *Salmonella* dissemination throughout the processing stages will occur (BUDIATI et al., 2013; BIBI et al., 2015).

Washing with hyperchlorinated water occurs during the first stage of fish processing, recommended at 5 ppm of free residual chlorine (BRASIL, 2009). This step aims at eliminating bacteria from the breeding environment and the mucus present on fish surfaces, which consists of glycoproteins released by the skin glands and is a culture medium for the proliferation of undesirable microorganisms. Thewash water should be at a low temperature, so as not to allow digestive enzymes to act before evisceration. Thus, at this stage, exposure time and chlorine concentrations should be adequate to eliminate pathogens present on fish surfaces (MACHADO et al., 2010).

The presence of *Salmonella* in the evisceration and descaling stages occurs due to operational failures, mainly due to viscera rupture, that exposes the meat to the bacterial contamination of gut contents, mainly ofe intestine, contaminating the handler's hands and disseminating the bacteria to other fishes. Considering that *Salmonella* may be present in the gastrointestinal tract, this is undoubtedly an important cross-contamination factor in fish slaughterhouses. In order to control cross-contamination, washing the intestinal cavity and the surface of the fish with hyperchlorinated water after the evisceration stage, should be performed, in order to eliminate blood and gut remains.

Salmonella antimicrobial resistance

A self-limiting gastroenteritis is the main clinical picture developed by *Salmonella*, which in severe cases may require replacement of liquid and electrolytes. The use of antibiotics is reserved for patients with serious diseases or at high risk of invasive diseases. Antibiotic therapy scheme for typhoid fever includes the third generation cephalosporin antibiotics, quinolones and macrolides. However, lately has been growing between typhoid *Salmonellas* and non-typhoid strains with high levels of resistance to quinolones and cephalosporin (COSBY et al., 2015). Emergence of multiple drug resistant to *Salmonella* (MDR) is currently a worldwide concern, and the occurrence of Salmonella MDR in food is a risky condition, representing an increase in the severity of Foodborne Disease, leading to increased hospitalization rates and possibility of death (CRUMP et al., 2015). Conversely, the epidemiology of antimicrobial resistance of *Salmonella* spp is complex and may be influenced by factors such as: antibiotic consumption, human travel, transmission between patients in hospitals, importation and trade of food of animal origin or not, trade in live animals in the country or between countries and exposure through an animal or human environment (ECDC/EFSA/EMA, 2017).

Salmonella resistant to antibiotics isolated from fish

In Morocco, 28 (49.1%) *Salmonella* isolates showed resistance to ampicillin (22 isolates), nalidixic acid (9 isolates), sulfonamide compounds (2 isolates) and tetracycline (1 isolates). Six isolates showed resistance to two antimicrobial substances (SETTI et al., 2009).

In a study conducted by COSTA et al. (2016), the antimicrobial susceptibility of 21 *Salmonella* strains obtained from Tilapia (*Oreochromis* spp.), both whole and in fillets, in the state of São Paulo was determined. Isolates were sensitive to gentamicin (95%), amikacin (66%) and ciprofloxacine (66%), and resistant to florfenicol (80%), which is surprising, since its use is relatively recent in veterinary medicine in Brazil.

A potential issue that has increased recently is the worldwide appearance of multidrug resistant phenotypes among *Salmonella* serotypes, such as *S.* Typhimurium and *S.* Enteritidis, especially resistance to quinolones, fluoroquinolones or cephalosporin.

CONCLUSION

Although, *Salmonella* is not part of natural fish microbiota, fish can become asymptomatic hosts, housing the bacteria mainly on the body surface and intestines, causing cross contamination in the industrialization and commercialization stages. The water quality and the aquaculture environment have a direct influence on microbiological fish contamination.

Most salmonellosis cases related to fish consumption in humans are caused by serovars *S.* Typhimurium and *S.* Enteritidis.The prevalence of *Salmonella* in freshwater fish ranges between 3.4 and 64%, related to water quality and good manufacturing practices (GMP).

During fish processing, the washing step with hyperchlorinated water should always be considered a CCP, since no further processing step can eliminate *Salmonella*. In this sense, the biggest challenge is to establish the best combination of chlorine exposure time vs. concentration required to inactivate the pathogen.*Salmonella* antibiotic-resistant strains have been isolated in fish in Brazil and worldwide, which evidences the transference of resistance genes among the aquatic microbial population, which can lead to more severe and difficult to treat foodborne infections.

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We have no conflict of interest to declare.

We have a competing interest to declare (please fill in).

SOURCES OF MANUFACTURES (optional)

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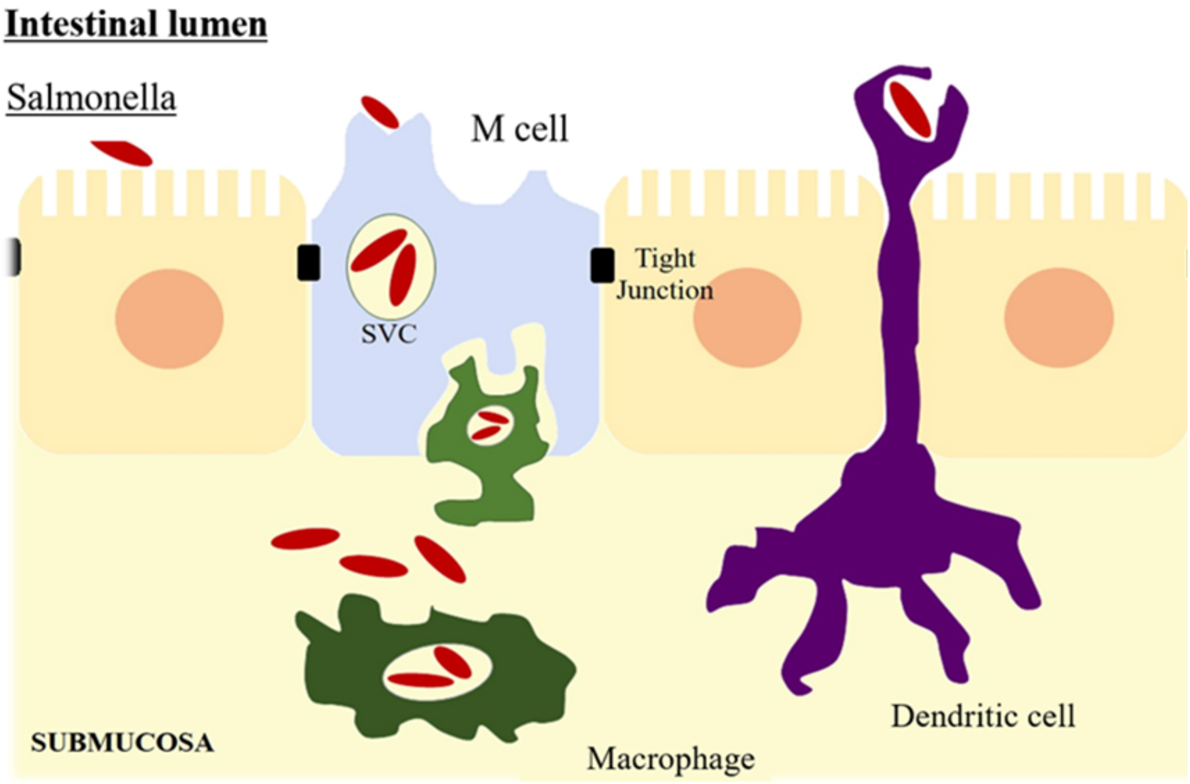
Table 1 - *Salmonella* prevalence in fish products.

Fish	Reared/ Captured	Prevalence %	Source	Samples (Positive)	Implicated serovars
Shrimp *(P. monodon)* Freshwater fish	Reared	49.1%; 36.6%	Vietnam	409 (84)	*S.* Typhimurium *S.* Anatum *S.* Weltevreden
Mussels (*Mytilus* spp.) Fish Shrimp (*Penaeus spp.)* Shellfish	Reared	27.2%; 12.4%; 4.6%; 6.0%	China	730 (217)	*S.* Aberdeen; *S.* Stanley; *S.* Wandsworth *S.* Typhimurium
Seafood[Octopus (*Octopus* spp.);Blue anchovy (*Pomatomus saltatrix)*] Freshwaters Fihes[Tilapia (*Tilapia rendalli*)]	Reared/ Captured	44.5%; 17.3%	United States	3840 (110)	*S.* Weltevreden *S.* Newport *S.* Senftenberg
Pangas (*Pangasianodon hypophthalmus*) Catfish *(Clarias gariepinus)* Tilapia *(Tilapia mossambica)* Rohu (*Labeorohita* spp.)	Reared	60%; 35%; 64%; 26.6%	Saudi Arabia	223 (89)	-
Shrimp (*P. brasiliensis*) Nile Tilapia (*Oreochromis niloticus*) Lobster tail (*Palinurus* spp.)	Reared	2.8%; 4.0%; 4.5%	Brazil	143(5)	-
Mussels (*Mytilus* spp.) Seawater sample Marine sediment	Captured	10% 4.1% 6.8%	Morroco	801 (57)	*S.* Blockley *S.* Kentucky *S.* Senftenberg
Silver carp (*Hypophthalmichthys molitrix*)	Reared	2.7%	Iran	39 (1)	-
Nile Tilapia (*Oreochromis niloticus*)	Reared	3.4%	Brazil	116 (4)	-
Mussels (*Mytilus* spp.) Oysters (*Ostrea edulis)*	Captured	3.1%; 2.5%	Spain	5,384(127)	*S.* Senftenberg *S.* Typhimurium



Legend: Types of cell invasion performed by *Salmonella*. Adapted from Sansonetti (2002).

Figure 1 – *Salmonella* pathogenicity mechanism in the Cell

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ORCID
Eduardo Eustáquio de Souza Figueiredo – (http://orcid.org/0000-0002-2608-4324)