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Review

Fish, Tilapia, and Shigellosis: A review

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Foodborne diseases are considered a relevant issue in health around the world due to their incidence, mortality and negative effects on the economic and productive sector. Fish is considered a food of high nutritional quality, being of global production, distribution and commercialization mainly for human consumption. Among the fish worldwide obtained from capture fisheries and mainly aquaculture for human consumption is Tilapia, due to the adaptability of this fish under cultivation conditions in addition to the fact that its meat is of quality and accessible economic value. Fish due to its composition, is highly susceptible to deterioration and contamination by different hazards throughout the food chain, putting the safety of products and public health at risk. Shigellosis is among the diseases that may be contracted from the consumption of food contaminated by bacteria of the genus *Shigella* spp.; food contamination is mainly related to inadequate or non-hygienic conditions and practices in the production, processing and handling of food. Therefore, the purpose of this review is to provide a general perspective of foodborne diseases, especially shigellosis, causal agents, conditioning factors, related foods such as fish, as well as control and preventive actions in order to protect the food safety and public health.

Key words: Food pathogens, food safety, food microbiology, enterobacteria, fisheries, aquaculture.

INTRODUCTION

The human being, through food, must consume adequate amounts of essential nutrients for the proper growth and development of his physical and intellectual capacities (Izquierdo et al., 2004). In addition, it has been considered that access to safe and nutritious food in sufficient quantity is essential to maintain life and promote good health (Tafur, 2009; Cortés-Sánchez et al., 2019; WHO, 2020b). Food safety is defined as the guarantee that food will not cause harm or illness to the consumer; this characteristic is considered a fundamental aspect of public health, in addition to the fact that, along with the nutritional, sensory and commercial characteristics, they constitute the total quality and wholesomeness of food (Fuertes et al., 2014; Jorquera et

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al., 2015; Cortés-Sánchez et al., 2019; Cortés-Sánchez et al., 2020).

Foodborne diseases are the product of the consumption of water and food contaminated with different physical, chemical or microbiological agents, which can be found naturally in food. These diseases constitute a relevant cause of mortality in developed and developing countries mainly, appearing at any time and place, prevailing in those where bad hygienic habits are practiced in the production, preparation, conservation and distribution of food, as well as in crowded conditions (Vásquez, 2003).

Throughout the food chain (from farm to table) it has been pointed out that there are various contaminating dangers of food, among them are those of biological origin, mainly bacteria, coming from commonly species of genera *Salmonella* spp.*, Shigella* spp.*, Escherichia* spp., *Listeria* spp., *Vibrio* spp., *Campylobacter* spp.*, Clostridium* spp.*,* among others) (Tafur, 2009; Palomino-Camargo, 2018; WHO, 2020b). Food is considered a good means of transport and growth environment for microorganisms due to its water and nutrient content, favouring this growth in hot climates, along with unhygienic product handling practices, pathogens can be transferred to food from many sources such as the soil, water, insects, animals, human beings, among others (Barbosa-Cánovas and Bermúdez-Aguirre, 2010). The presence of these different microorganisms in food can put the health of consumers at risk and trigger diseases considered a serious problem in the health sector worldwide due to their incidence, mortality rate, as well as their negative effects on the social and economic development (Tafur, 2009; Palomino-Camargo, 2018; WHO, 2020b).

Nowadays, the globalization of markets has led to processes of supply and exchange of food products, either fresh or processed, between different countries; therefore, the health and food safety issues are considered essential, and cooperation between different entities such as governments, producers and consumers contribute to guaranteeing food safety and public health (Tafur, 2009; WHO, 2020b). The purpose of this review is to provide a general perspective of foodborne diseases, especially Shigellosis, its causal agents, conditioning factors, foods related to its transmission such as fish and tilapia, control and prevention actions in order to protect food safety and public health.

FOODBORNE DISEASES

Foodborne diseases are considered around the world an important public health problem due to their incidence, mortality and the negative effects on socio-economic aspects due to the decrease in productivity, trade and expenses in health systems for hospitalizations and medications, in addition to economic losses to the food industry because of contamination and deterioration of products (Vázquez, 2003; Barrantes and Achi, 2011; Alerte et al., 2012; López et al., 2013; Jorquera et al., 2015; Hernández et al. , 2016; Soto et al., 2016; WHO, 2020).

Foodborne diseases are caused by intentional or accidental consumption of water and food contaminated by microorganisms or chemical substances (Vásquez, 2003; WHO, 2020). The most common symptoms of these diseases are gastrointestinal such as nausea, vomiting, diarrhea, abdominal pain and fever, and presenting, in some severe cases, complications such as sepsis, meningitis, abortions, Reiter's Syndrome, Guillan-Barré Syndrome, cancer, or death (López et al., 2013; Soto et al., 2016; WHO, 2020). Globally, it is estimated that these diseases affect one in 10 people, and that 420,000 people die a year, especially children under 5 years of age (WHO, 2020a). For these diseases, population groups with greater risk and affectation have been considered where, in addition to children under 5 years, are people in poverty, pregnant women, the elderly, and people with weakened immune systems (Alerte et al., 2012; Soto et al., 2016). About 250 agents that cause foodborne diseases have been described and classified, including: chemical agents (inorganic compounds, antimicrobials, toxic food additives, toxins, lubricants, inks, disinfectants, heavy metals, pesticides), physical agents (glass shards, metal , wood, or others), and biological (bacteria, parasites, viruses and prions), the latter being particularly, bacteria such as *Clostridium* spp., *Bacillus* spp.*, Vibrio* spp.*, Aeromonas* spp., *Salmonella* spp., *Campylobacter* spp., *Shigella* spp., *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli,* main responsible for cases and outbreaks (Alerte et al., 2012; Jorquera et al., 2015; Rodríguez et al., 2015; Cortés-Sánchez,2016; Soto et al., 2016; Cortés-Sánchez et al., 2019).

Bacteria that cause foodborne diseases are classified: 1) Foodborne infections, which occur when a pathogen is present in the food that establishes and multiplies in the consumer, and which present two variants: a) invasive infections, where the microorganism invades, colonizes tissues and organs of the infected person. Within this group: *Salmonella* spp.*, Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., and entero-invasive *E. coli* (EIEC), and b) Toxi-infections, caused by non-invasive bacteria capable of colonizing and multiplying themselves in the intestinal tract of the host, synthesizing and excreting toxins. Some bacterial toxin producers: *Vibrio parahaemolitycus*, *Vibrio cholerae, Bacillus cereus* (enterotoxin-producing strains), *Clostridium botulinum, Clostridium perfringens*, and enteropathogenic variants of *E. coli* (Kopper et al., 2009; Cortés-Sánchez et al., 2015; Rodríguez et al., 2015). 2) Foodborne intoxication colloquially referred to as food poisoning, derived from the consumption of food containing toxins produced by bacteria that have contaminated and grown in the food at a certain concentration. Some examples are: *Clostridium*

botulinum, B. cereus (strains that produce the emetic toxin), and *Staphylococcus aureus*. It should be noted that, in the diagnosis and differentiation with infections or general intoxications, they show a more rapid clinical manifestation (Kooper et al., 2009; Rodríguez et al., 2015).

The presence of these diseases can be a direct indicator of the hygienic quality of the food available for consumption, where the contamination of these can occur throughout their production, processing or by the use of contaminated raw material, because multiple pathogenic bacteria can form part of the normal microbiota of animals destined for human consumption such as poultry, pigs, cattle, among others (Cortés-Sánchez et al., 2015). The global incidence of food-borne diseases has been associated with the sum of several factors such as market globalization, climate change, introduction of new products and manufacturing processes, migration, changes in eating habits with the growing demand for ready-to-eat foods, as well as the consumption of food and water outside the home contaminated mainly due to the lack of hygiene in the preparation of food, and the absence in many occasions of water treatment in food preparation (Jorquera et al., 2015; da Cunha et al., 2017; López et al.,2020).

Shigella

Bacilli of the genus *Shigella* spp., belong to the *Enterobacteriaceae* family, which are short bacilli of 1-3 µm, Gram negative, do not form spores, immobile, facultative anaerobes, do not ferment lactose but do glucose to acid where they do not typically produce gas, do not hydrolyze urea, do not produce hydrogen sulphide, do not decarboxylate lysine, and do not use citrate and sodium acetate as the only carbon source (Acha and Szyfres, 2001; Torres, 2004; Álvarez et al., 2008; Lampel, 2009; Puerta-García and Mateos-Rodríguez, 2010; da Cunha et al., 2017), and like other *Enterobacteriaceae*, they can be universally located in soil, water and vegetation, as well as in the gastrointestinal system of animals and human beings (Puerta-García and Mateos-Rodríguez, 2010). These bacteria grow at temperatures between 10 and 48°C but do not grow at temperatures ≤ 7°C, they have an optimal growth pH between 6 and 8, although they can survive in acidic pH (5.5) and low relative humidity, NaCl concentrations of 4 to 5%, have a resistance to heat of 60°C / 5 min, and a survival in water up to six months (Huss, 1997; Elika, 2013; da Cunha et al., 2017).

The genus *Shigella* spp*.,* presents four species, and each one of them have several serotypes according to somatic antigen "O", being: *Shigella dysenteriae* (serogroup A, 15 serotypes), *Shigella flexneri* (serogroup B, 8 serotypes), *Shigella boydii* (serogroup C, 20 serotypes), and *Shigella sonnei* (serogroup D, one serotype), all of them having epidemiological importance (Romero, 1999; Acha and Szyfres, 2001; Álvarez et al., 2008; Lampel, 2009). These bacteria are of worldwide distribution and importance in human health: *Shigella dysenteriae* (Africa, Asia and Central America), *S. flexneri* (Developing countries), *S. sonnei* (developed countries), and *S. boydii* (India and developed countries) all responsible for Shigellosis, a disease transmitted by contaminated water and food whose severity and lethality depend on the host (age and nutritional status) and causal species (Acha and Szyfres, 2001; León-Ramírez, 2001; Torres, 2004; Lampel, 2009; Puerta-García and Mateos-Rodríguez, 2010; Barrantes and Achi, 2011; Elika, 2013; Ranjbar et al., 2014; da Cunha et al., 2017; CDC, 2020). The infection has an incubation period of 1 to 2 days after consuming contaminated food with a duration of 5 to 7 days of illness (FDA, 2018; CDC, 2020). The disease involves inflammation of the gastrointestinal tract, fever, diarrhea with blood and mucus, sometimes vomiting and headache, while in severe cases complications such as intestinal perforation, reactive arthritis, and Hemolytic-Uremic Syndrome (HUS) can occur as well (León-Ramírez, 2001; Acha and Szyfres, 2001; Torres, 2004; Lampel, 2009; Puerta-García and Mateos-Rodríguez, 2010; Barrantes and Achi, 2011; Elika, 2013; Ranjbar et al., 2014; da Cunha et al., 2017; CDC, 2020).

Shigellosis presents as its main vulnerable groups the elderly, people with weakened immune systems, and children under 5 years of age (Huss, 1998; da Cunha et al., 2017; CDC, 2020). In the latter group, Shigellosis makes an important contribution to the infant mortality rate estimated worldwide at approximately 28,000 children each year (Lluque et al., 2015). Where, *S. dysenteriae* is considered the cause of large and prolonged epidemics of Shigellosis or dysentery and its infection the more serious and lethal than other groups of *Shigella* (Acha and Szyfres, 2001; León-Ramírez, 2002; Mindy et al., 2004).

The treatment of *Shigella* infections involves reducing water and electrolyte disorders and the use of antibiotics such as ampicillin, tetracyclines, erythromycin, azithromycin, sulfamethoxazole-trimethoprim, and quinolones in severe cases (Romero, 1999; León-Ramírez, 2002; Álvarez et al., 2008; Lluque et al., 2015; da Cunha et al., 2017; CDC, 2020). However, an increase in the incidence of antimicrobial resistance has been reported by isolates of *Shigella* spp., of clinical origin and food, including fish, considering the phenomenon of resistance in food pathogens a global health problem and the development of a *Shigella* vaccine is taken as a high priority (Guchi and Ashenafi, 2010; Lluque et al., 2015; Hosseini et al., 2016; Ogbonna and Inana, 2018; Noor et al., 2020). The species of the genus *Shigella* have a phylogenetic relationship with the genera *Salmonella* and *Escherichia*, but unlike these, it does not have animal reservoirs, being detected only in

humans and in some cases primates that can be considered accidental hosts, meaning that there is a risk of zoonotic transmission, in addition to its presence in the environment being related to faecal contamination (Huss, 1997; Acha and Szyfres, 2001; Lampel, 2009; Elika, 2013; da Cunha et al., 2017). Among the pathogenicity mechanisms of these bacteria are the fimbriae that intervene in adhesion phenomena, antigens of the invasive plasmid (Ipa) that is directly related to the infectious process (entry) to the host cell to colonize intestinal mucosa epithelium, endotoxins (lipopolysaccharide wall), enterotoxins (ShET-1 and ShET-2), cytotoxins or shiga toxins (VT-1 and VT2) (Romero, 1999; Leon-Ramirez, 2002; Madigan et al., 2004; Ausina and Moreno, 2005; Álvarez et al., 2008; Polifroni et al., 2009).

Shigella species are highly transmissible, having a low infectious dose of around 10 to 200 cells that can be transmitted through the faecal-oral route by direct contact due to lack of hygiene from person to person, and by consumption of food or water contaminated with human faeces, derived from inadequate hygiene practices in the preparation and handling (infected or asymptomatic food handlers, contaminated equipment and surfaces), or by contamination by vectors such as insects specifically flies (Huss, 1998; Leon-Ramirez, 2002; Álvarez et al., 2008; Lampel, 2009; Elika, 2013; Hernández, 2016; da Cunha et al., 2017; FDA, 2018; CDC, 2020). The most commonly implicated foods in the transmission and cases of Shigellosis are raw or insufficiently heat-treated foods such as raw fruits and vegetables consumed in salads, raw milk and dairy products, raw fish and oysters, beef, chicken, and drinking contaminated water (Huss, 1998; FAO, 2009; Elika, 2013; FDA, 2018).

FISH

Fish is defined as any food that can be extracted from oceanic or continental waters (fresh or brackish) destined for human or animal consumption (Soares and Gonçalves, 2012). Fish is a food commercialized around the world, highly nutritious, and an important constituent of the human diet since it is a source of polyunsaturated lipids, vitamins, minerals, and proteins with a high biological value and digestibility (Soares and Gonçalves, 2012; Fuertes et al., 2014; FAO, 2020). Fish has been estimated to be one of the main suppliers of animal protein for human consumption around the world, supplying 25% of animal protein in developed countries, and over 75% in developing countries (Baltazar, 2007; Romero et al., 2015). Fish destined for human consumption comes from capture fishing and aquaculture activities where, according to a FAO total world production estimate in 2018 from both activities reached 178.5 million tons, with a per capita consumption worldwide of 20.5 kg (FAO, 2020).

On the other hand, in addition to the high nutritional contribution, production, distribution and global consumption of fish, it should be noted that it is a food with high susceptibility to contamination, deterioration and short commercial life, due to intrinsic properties such as pH close to neutrality, high water activity, and nutrient content easily usable by microorganisms, which leads to economic losses for the food industry (Esteveao and Pucci, 2000; Soares and Gonçalves, 2012; Romero et al., 2015). Foods with these characteristics constitute a favourable substrate for the development of micro-organisms, where a minimal microbial contamination, coupled with an adequate incubation temperature and time, makes it a food of high risk of diseases to consumers (Kopper et al., 2009).

Fish contamination can occur at any stage of the food chain by various microorganisms or chemical agents through water, soil, air, fauna, ingredients, utensils, humans, processing or storage conditions, representing a high risk to public health due to the transmission of diseases (Hernández, 2010; Fuertes et al., 2014; WHO, 2020). Fish after fishing and harvesting can be altered and lose its quality through different processes such as autolysis, oxidation and microbial activity (Huss, 1998; Fuertes et al., 2014). In addition, that factors such as species, age, the medium they live in, feeding, conditions of capture, handling, processing, conservation, conditions of transport, distribution and commercialization influence the nutritional value, quality and safety of the fish (Huss, 1998; Soares and Gonçalves, 2012; Fuertes et al., 2014). The microbial activity in fish is relevant in aspects of deterioration and safety, where various members of the fish microbiota are related (Huss, 1998; Fuentes et al., 2011; Romero et al., 2015). Microorganisms are found on all external surfaces - skin and gills - and intestines of live fish. The total number of microorganisms varies enormously, establishing a normal range of 10^2-10^7 / cm^2 on the surface of the skin, and for gills and intestines between 10^3 and 10^9 CFU / g (Huss, 1999). Bacteria found on the skin and gastrointestinal content of living fish do not invade the muscle or meat, it is sterile due to the protection of its natural defenses, but when the fish dies the bacteria penetrate into the fish (Romero-Jarero and Negrete-Redondo, 2011; Romero et al., 2015). Within the microbiota of fish there are predominantly Gram-negative bacteria belonging to the genus *Pseudomonas* spp., *Moraxella* spp., *Acinetobacter* spp., *Shewanella* spp., *Flavobacterium* spp., *Vibrio* spp., *Photobacterium* spp., and *Aeromonas* spp., followed by Gram positives ones such as *Bacillus* spp., *Micrococcus* spp., *Clostridium* spp., *Lactobacillus* spp., and *Corynebacterium* spp. (Huss, 1999; Romero et al., 2015). These microorganisms have been associated with the deterioration of fish, and their presence and activity are a function of the nature and conditions of the aquatic environment, where temperature is considered a selective factor (Huss, 1999; Romero-Jarero and Negrete-Redondo 2011; Soares and Gonçalves, 2012; Romero et al., 2015).

The loss of quality and deterioration of fish are especially a consequence of bacterial activity including pathogens (Esteveao and Pucci, 2000). The deterioration of the fish can be perceptible sensorily, through presence of odours derived from the bacterial, and use of small and water-soluble molecules in the fish tissue such as non-protein nitrogen (amino acids, small peptides and trimethylamine oxide), alteration of chain fatty acids short (lactic acid, butyric acid), tissue lipid aldehydes and ketones, volatile sulphides (hydrogen sulphide, dimethyl sulphide, methyl mercaptan), amines (indole, skatole), biogenic amines (histamine, putrescine, and cadaverine), and ammonia from amino acids or metabolized proteins (Romero et al., 2015).

Within aspects of food safety, the pathogenic bacteria present as part of the fish microbiota, and the risk of transmitting diseases to the consumer, are classified into two groups: 1) Autochthonous, widely distributed in aquatic environments around the world, where the temperature of the water presents a selective effect. Some of these microorganisms are: *Vibrio* spp*., Clostridium botulinum, Aeromonas hydrophila, Plesiomonas shigelloides,* and *Listeria monocytogenes*; and 2) Non-autochthonous, consisting of different *Enterobacteriaceae* such as *Serratia* spp., *Citrobacter* spp*., Proteus* spp*., Salmonella* spp*., Shigella* spp*., Enterobacter cloacae, Escherichia. coli*, among others, as well as Gram positive ones such as *S. aureus, S. epidermidis, S. agalactiae, S. pyogenes*, found in fishery products due to faecal contamination from the animal and / or human reservoir of natural waters or aquatic environments and direct contamination of the products during their processing, conservation and handling (Huss, 1998, 1999; Romero-Jarero and Negrete-Redondo 2011; Cortés-Sánchez et al., 2019).

Fish consumption has been associated with cases and outbreaks of diseases around the world, having mainly as causative agents' various bacterial pathogens such as *Campylobacter jejuni, E.coli, Listeria monocytogenes, Clostridium* spp., *Salmonella* spp*., Vibrio* spp*., Streptococcus* spp*., Shigella* spp., and *Staphylococcus* spp. (Soares and Goncalves, 2012; Alerte et al., 2012; Espinosa et al., 2014; Soto et al., 2016). Therefore, fish can be considered a food of sanitary risk as it is a carrier of pathogenic bacteria, either as part of its microbiota or as a consequence of the contamination that can occur in phases of capture, cultivation, processing, handling, conservation, transport, elaboration, and preparation (Esteveao and Pucci, 2000; Romero-Jarero and Negrete-Redondo 2011; Fuentes et al., 2011; Espinosa et al., 2014).

Tilapia

Various species of fish of the *Cichlidae* family are known by the name of Tilapia, which belong to the genus *Sarotherodon, Oreochromis* and *Tilapia*, thus classified according to their parental care behaviour (Baltazar, 2007; Amal and Zamri-Saad, 2011; Jácome et al., 2019). These freshwater fish are of African origin and are highly relevant in the production of animal protein in tropical and subtropical waters around the world, especially in developing countries (Baltazar, 2007; INP, 2018; Jácome et al., 2019).

The genus of greatest interest in aquaculture activities is the *Oreochromis* (*O. niloticus, O. mossambicus,* and *O. aureus*), and hybrids, because of their high growth rates, rapid growth, resistance to diseases, adaptation to captivity, acceptance of feeding diets, and for having good quality meat at an affordable price (Baltazar, 2007; Jácome et al., 2019). Tilapia is one of the finfish with the highest aquaculture production in the world with 10.2% of total production, being the *Oreochromis niloticus* species the most produced in Asian countries, having China among the largest producers (FAO, 2020).

In Latin America, countries like Mexico produce Tilapia mainly through the aquaculture of several species such as: a) *Tilapia rendalli* (herbivorous Tilapia), b) *Oreochromis niloticus* (Nile Tilapia), c) *O. niloticus* Variety Stirling (Stirling Tilapia), d) *O. niloticus* Rocky Mountain Variety (White Tilapia, Blue Tilapia), e) *O. aureus, O. mossambicus*, (Mozambique tilapia), f) *O. mossambicus* Orange Variety (Orange Tilapia), g) *O. urolepis hornorum* (Tilapia mojarra), and h) *Oreochromis* sp. *(O. mossambicus x O. urolepis hornorum*) Florida red Tilapia (hybrid) (INP, 2018; SAGARPA, 2018). The cultivation systems can be extensive, intensive, and semi-intensive according to the cultivation density, food supply and type of cultivation system (INP, 2018). Mexico is considered the fifth largest producer worldwide (SAGARPA, 2018). These fish are cultivated in almost the entire national territory, being the states with the highest production: Chiapas, Jalisco, Nayarit, Sinaloa, Tabasco, Hidalgo, Guerrero, Estado de Mexico, and Veracruz (SAGARPA, 2018; INP, 2018). Finally, this food is marketed with an average weight of 250 - 300 g as fresh whole gutted, whole frozen gutted and processed into fresh or frozen fillet, reaching an annual per capita consumption of 2 kg (INP, 2018).

In aspects of quality, deterioration and food safety, the microbiota of fish and tilapia is relevant and is related to that existing in the waters and habitat from which it comes, temperature, depth and degree of contamination of the waters. Highlighting the presence of Gram-negatives such as: *Pseudomonas* spp., *Shewanella* spp., *Moraxella* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Vibrionaceae* and *Aeromonadaceae*. Gram positive there is a variable proportion of *Bacillus* spp., *Micrococcus* spp., *Clostridium* spp., *Lactobacillus* spp., and *Corynebacterium* spp. Bacteria of sanitary interest and contamination indicators members mainly of the *Enterobacteriaceae* family, including *Shigella* spp., are found in fish from waters highly contaminated with faecal

matter or subjected to inadequate hygiene conditions during handling or processing (Morales et al., 2004; Onyango et al., 2009; Awuor et al., 2011; Fuentes et al., 2011).

CONTROL, PREVENTION, AND SANITARY REGULATION OF FOOD IN THE CONTEXT OF SHIGELLOSIS

The quality and safety of food products are aspects of relevance in public health, which is evidenced by the development of quality regulations around the world in the different phases of the production chain (Soares and Goncalves, 2012). Generally for fish and fishery products, a large part of the associated foodborne diseases are derived from the lack of good hygiene and handling practices of the fish after capture, as well as the contamination of the fish with pathogenic microorganisms in the primary production stage (aquaculture) due to exposure to harmful microorganisms present in human or animal excreta (WHO/FAO 2016).

International organizations as the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), have developed different codes in order to achieve the objective of making available food safe and suitable for human consumption. Among the codes developed are the essential general principles of food hygiene applied throughout the food chain "CAC / RCP 1-1969" (FAO / WHO, 2020).

Also, the code of practice for fish and fishery and aquaculture products "CXC 52-2003". Its purpose is to provide basic information for the development of fish and shellfish process management systems that incorporate Good Manufacturing Practices (GMP), as well as the application of Hazard Analysis and Critical Control Points systems (HACCP) (FAO / WHO, 2020). It has also been generally established that the actions and key points for the safety of aquaculture products are the hygiene of personnel, equipment and materials for capture or handling, cleaning of ponds, fish health and water quality (WHO / FAO 2016).

Likewise, FAO has developed guidelines for risk-based fish inspection that provide technical information to support fish inspection systems on food safety aspects in order to guarantee consumers access to safe and nutritious fish, in addition to helping to develop, harmonize and apply preventive inspection systems, as well as to guide the development of regulatory frameworks for fish inspection in developing member countries (FAO, 2009).Within the food safety regulations developed for fishery and aquaculture products in European countries such as Spain, it has been through the Order of August 2, 1991, where the standards and microbiological limits for *Shigella* spp., were established, which must be absence of Salmonella-Shigella in 25 g of sample of fresh, salty, refrigerated, frozen, dry, salty, salty and dry, semi-pickled and smoked products (BOE, 1991). However, this provision was repealed by Royal Decree 135/2010, of February 12, 2010 by transposition of community regulations, such as Regulation (EC) No. 2073/2005, relating to microbiological criteria applicable to food products (BOE, 2010).

The legislation on food safety in the European community for the production, acceptability and commercialization of food, can be found in the Regulation (CE) No. 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority, and laying down procedures in matters of food safety. Regulation (EC) No 852/2004 on the hygiene of food products that includes the general application of procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP) that, along with the application of correct hygienic practices, reinforce the responsibility of food business operators. Regulation (CE) No 853/2004 establishes specific hygiene rules for food of animal origin, including fish and products. Regulation (EC) No 854/2004 establishes specific rules for the organization of official controls of products of animal origin intended for human consumption. Regulation (EC) No 882/2004, carried out on official controls, to guarantee the verification of compliance with the legislation on feed and food and the regulations on animal health and animal welfare, and Regulation (EC) No 2073/2005 which establishes the microbiological criteria applicable to food products including fish and its various biological hazards, providing guidance on the acceptability of food products and manufacturing processes (EC, 2020). However, the latter does not establish specific microbiological limits for food and its contamination by *Shigella* (Elika, 2013).

In Latin American countries such as Mexico, sanitary regulation regarding fish safety through the food chain begins in primary production, with the implementation of good aquaculture practices during primary fish production and the implementation of good aquaculture practices specifically in Tilapia production to ensure food safety (García and Calvario, 2008; SENASICA, 2019). Likewise, the regulatory framework is made up of the Official Mexican Standard "NOM-242-SSA1-2009" for fresh, refrigerated, frozen and processed fish products, which include the sanitary specifications and test methods for these products, although for *Shigella* spp., no sanitary specification is established in these foods, and it has been suggested as a quality criterion for raw foods of <1 CFU / 25g of sample (Hernández, 2016). The “NOM-251-SSA1-2009” standard, which establishes the minimum requirements of good hygiene practices that must be observed in the process of food, beverages or food supplements, and their raw materials in order to avoid their contamination throughout their process; it, also establishes the Hazard Analysis and Critical Control Point (HACCP) system and guidelines for its application. For the specific case of the fish product processing industry, the "NOM-128-SSA1-1994" standard establishes the application of a system HACCP in the industrial

processing plant. The “NOM-210-SSA1-2014” standard establishes the general and alternative test methods for the determination of microbial and pathogenic indicators in food for human use and consumption, although it does not establish specific methods for *Shigella* spp., the “NOM-230-SSA1-2002” standard, which specifies the sanitary requirements that public and private supply systems must meet for the management of water intended for human use and consumption. And the “NMX-F-605-NORMEX-2016” standard that establishes the provisions of good hygiene and sanitation practices that food and beverage service providers must comply with to obtain the distinctive “H”.

In general, actions for the control and prevention of foodborne diseases such as Shigellosis should be all along the food chain, from primary production, handling, processing or transformation of food, to consumption (Figure 1), which is achieved through the implementation of conditions hygienic and sanitary, Sanitation Standard Operation Procedure (SSOP), good hygiene practices with an emphasis on hygiene training and information for food handlers (detection and control of symptomatic and asymptomatic carriers), microbiological criteria of raw materials and final products for their acceptability, operational temperature control where the food must be subjected to heat treatments above 60°C, as well as to preserve the cold chain during transport, storage and distribution of raw foods susceptible to contamination, and finally the strengthening of all the previous measures with control systems based on the Hazard Analysis and Critical Control Points (HACCP), and traceability systems for the inspection, certification and sanitary control of products throughout the food chain (Huss, 1998; Leon-Ramirez, 2002; CAC/GL 60-2006; Lampel, 2009; Bracho et al., 2012; Elika, 2013; Hernandez, 2016; da Cunha et al., 2017; Palomino-Camargo, 2018; PAHO, 2020).

While the actions at the level of manipulation and end consumer in homes are suggested to being carried out with good hygiene and manipulation practices in the preparation and cooking of food to prevent contamination, the World Health Organization has developed manuals to promote safety of food in this context through the cleaning and hygiene of food preparation areas, handlers, equipment and utensils to be used, to avoid cross contamination (raw and cooked food), to avoid the presence of vectors such as flies, favour conditions of time and temperature in the preparation (> 60°C), and preservation of food (<5°C), as well as to have a good sanitary quality of the water used (OMS, 2007; Elika, 2013; Hernandez, 2016).

Food analysis in the laboratory

As part of the control and prevention of diseases through food, government sanitary agencies and the food industry, in addition to hygiene education, inspection of facilities and activities, consider microbiological analysis (Huss, 1997). The objective of microbiological analysis in foods such as fish is the detection of pathogens, indicators of faecal contamination, inadequate or poor processing conditions, and practices in hygiene aspects, to ensure the quality is at an adequate microbiological level (Huss, 1998).

The detection of pathogens in food such as *Shigella* spp., is generally carried out by conventional cultivation because its execution and costs are accessible. These methods are based on phenotypic characteristics of microorganisms such as their morphology, development, biochemical, metabolic, and antigenic properties (Table 1) (Prats and Mirelis, 1998; Barrantes and Achi, 2011; Bou et al., 2011; da Cunha et al., 2017).

In traditional food microbiological analysis methods, it must be considered that *Shigella* is sensitive to environmental conditions and can die quickly, particularly when exposed to direct sunlight or food processing conditions (dehydration), intrinsic characteristics of the food (composition, pH and saline concentration), in addition to the natural microbiota that competes for nutrients, affect and complicate the isolation of the pathogen from food, so that the analysis may require enrichment methods for better detection when it is in low numbers or weakened physiological state (Lampel, 2009; Anselmo et al., 2020).

In traditional culture, various differential and selective culture media are used for the isolation and detection of *Shigella* spp.; the most used are Eosin Methylene Blue Agar (EMB Agar), Mac Conkey agar, and the Salmonella-Shigella agar (SS), where typical colonies are colourless or transparent (because lactose is not fermented), the Xylose-Lysine-Deoxycholate (XLD) agar, where colonies are transparent (no xylose fermentation or lysine modification), the Hektoen agar, where colonies are transparent green (no lactose fermentation and no hydrogen sulphide production), and the Tergitol 7 agar, where *Shigella* colonies appear blue (lactose negative). Suspicious colonies are inoculated onto Kligler's media (KIA) or Triple Sugar Iron agar (TSI) and Lysine Iron Agar (LIA). Any colony that ferments glucose in these media without producing gas, does not use lactose, does not produce H_2S, is immobile, does not decarboxylate and deaminates lysine, does not deaminates tryptophan, in addition to not having urease or oxidase production is suspected *Shigella* spp. (Prats and Mirelis, 1998; León-Ramirez, 2002; Mindy et al., 2004; Álvarez et al., 2008; Lampel, 2009; Wallace and Andrew, 2018; Anselmo et al., 2020). The biochemical identification and differentiation tests of bacteria can be manual or automatic, having commercially available manual multiple test systems to API (bioMérieux), Enterotube (BBL), Oxi / Ferm Tube (BD), RapID and MicroID systems (Remel), biochemical ID systems (Microgen). While the automated multiple test systems available are MicroScan, ATB, Pasco, Wider, Phoenix, Vitek among others (Bou et al.,

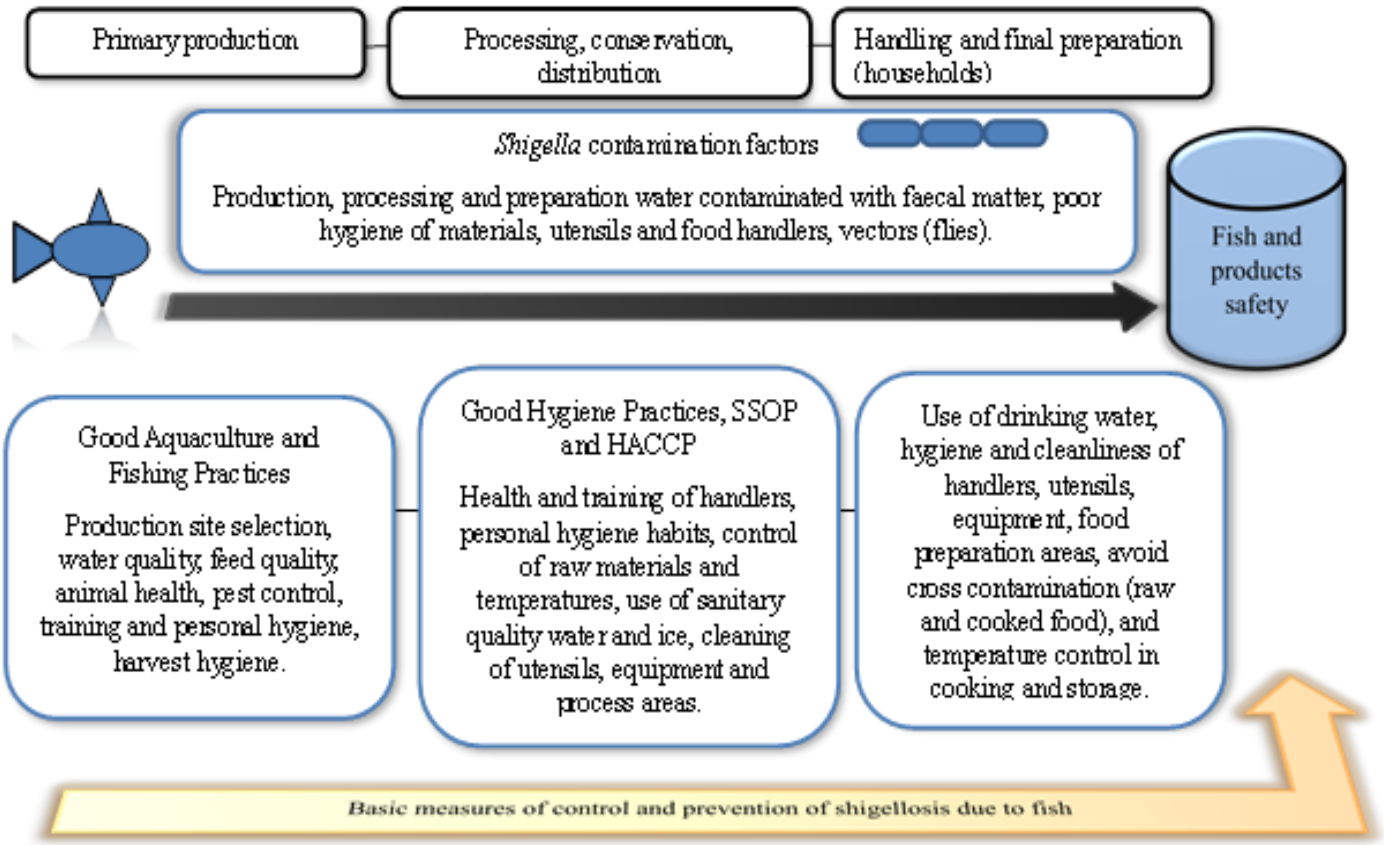

Figure 1. General scheme of contaminating factors caused by *Shigella* and prevention actions in the food chain of fish and products. SSOP: Sanitation Standard Operation Procedure.

2011). In addition to serological identification by agglutination with specific antisera (Prats and Mirelis, 1998; León-Ramirez, 2002; Mindy et al., 2004; Álvarez et al., 2008; Lampel, 2009; Wallace and Andrew, 2018; Anselmo et al., 2020).

Various standardized methods have been developed for the isolation and identification of *Shigella* spp., in food matrices. Some of these methods are that of Wallace and Andrew (2018), present in the Bacteriological Analytical Manual (BAM) of the U.S. Food and Drugs Administration (FDA), where through a traditional culture method that uses a selective enrichment phase where 25 g of sample are placed and homogenized in 225 ml of *Shigella* broth added with novobiocin (0.5 to 3 µg / ml), the pH is adjusted to 7 and incubated under anaerobic conditions at 44°C (for *S. sonnei*) and 42°C (for the rest of *Shigella* species), then the enrichment culture suspension is shaken and sub-cultured on Mac Conkey agar incubating for 20 h at 35°C, and subsequently analyzing the growth of typical colonies (slightly pink and translucent, with or without rough edges). The colonies are selected for confirmation through different biochemical tests (glucose broth, TSI agar, lysine broth decarboxylase, motility agar and tryptone) and serological identification tests.

Another standardized traditional microbiological analysis method is the one developed by the International Organization for Standardization (ISO) ISO 21567:2004, where the enrichment is done in *Shigella* broth with novobiocin, incubated at 41.5± 1°C from 16 to 20 h in anaerobic conditions. Afterwards, varied differential and selective culture media, such as Mac Conkey agar, XLD agar, and Hektoen agar, are inoculated and incubated at 37°C for 20h to 24 h. Colonies with typical morphology are subjected to biochemical confirmation tests using Triple Sugar Iron agar (TSI), motility agar, urea, lysine decarboxylase, ornithine decarboxylase, indole formation, detection of β-galactosidase, and the use of different carbohydrates and as additional acetate, mucate and citrate tests (Figure 2) (ANMAT-RENAPRA, 2013).

However, despite being the traditional microbiological methods of frequent and generalized use in the isolation and identification of pathogens in food, these can present drawbacks, such as the absence of correlation between the morphological or phenotypic characteristics of the isolate analyzed, and that corresponding to the strain or type species used, the analysis time and the results obtained may take days or weeks, they may present low sensitivity, in addition to the fact that some bacterial cells

Table 1. Biochemical identification characteristics of different *Enterobacteriaceae* (Pelczar, 1982; Madigan et al., 2004).

Test	*Shigella* spp.	*Escherichia* spp.	*Salmonella* spp.	*Klebsiella* spp.
Oxidase	-	-	-	-
Nitrates reduction	+	+	+	+
Arginine	-	V	+	-
Gelatin Hydrolysis	-	-	V	V
Alanine deaminase	-	-	-	-
Acid production of: Adonitol	-	-	-	V
Inositol	-	-	V	+
Carbon source: Citrate	-	-	+	V
Malonate	-	-	V	V
Tartrate	-	+	V	V
Voges Proskauer	-	-	-	V
Methyl Red	+	+	+	V

V = variable reaction, - negative reaction, + positive reaction.

may enter a Viable But Non-Culturable state (VBNC) that consists of a state of survival to adverse conditions (food processing) and that most *Shigella* species such as *S. dysenteriae* can present and the method used may not be efficient (Ishrat et al., 1996; Wen-Bin et al., 2007; Barrantes and Achi, 2011; Bou et al., 2011; Palomino-Camargo and González-Muñoz, 2014; Huertas-Caro et al., 2019).

To try to avoid the drawbacks of traditional microbiological analysis methods, alternative methods have been developed for the detection of food pathogens based on the Polymerase Chain Reaction (PCR) and its multiple variants; which includes the detection of different targets of genetic material that include housekeeping genes, genes that encode virulence factors, as well as the Whole Genome Sequencing (WGS) by Single Nucleotide Polymorphism (SNP) of the whole genome, markers and k-mers. In this way, for food safety, epidemiological or phylogenetic studies, a greater number of samples can be analyzed, and the results are obtained with greater speed and sensitivity (Barrantes and Achi, 2011; Shao et al., 2011; Palomino-Camargo and González-Muñoz, 2014; Ragupathi et al., 2018; Huertas-Caro et al., 2019).

For *Shigella* spp., various methods have been developed using virulence regions or factors for its detection, such as: the *ial* region (invasion associated locus) present in the invasion plasmid, whose product is 320 bp and is encoded in the SSTT spa´s maximum operon (essential in the invasive phenotype) (Barrantes and Achi, 2011), Shiga toxin gene (*Stx*) (Ishrat et al., 1996; Villacrés et al., 2015), enterotoxin 1 (ShET-1), encoded protein in the bacterial chromosome by genes *set1A* (subunit A of the protein) of 309 bp and *set1B* (subunit B of the protein) of 147 bp (Villacres et al., 2015), insertion sequence 1 (IS1) 768-bp DNA fragment (Wen-Bin et al., 2007), 215 bp *virA* gene (Villalobo and Torres, 1998; Alipour et al., 2012), *icsA* gene encodes the IcsA protein, for intercellular mobility and dispersion (Alipour et al., 2012), and plasmid invasion antigen “H” or *ipaH* gene of 610 bp specific for *Shigella* spp., being determined as a stable gene to be detected because it is found in the chromosome and in plasmid DNA in multiple copies (Pichel and Caffer, 2016; Wang et al., 1997; Thiem et al., 2004; Lampel, 2009; Barrantes and Achi, 2011; Mokhtari et al., 2013; Radhika et al., 2014; Lin et al., 2010; Ranjbar et al. 2014; Villacrés et al., 2015; Nadella et al., 2017).

Likewise, molecular subtyping methods such as Pulsed-Field Gel Electrophoresis (PFGE) and Multilocus Variable-Number Tandem Repeat Analysis (MLVA) have been incorporated to determine the genetic relationships between isolates of *Shigella* spp., which contribute to epidemiological surveillance aspects, identification, and study of outbreaks caused in different geographical regions by the pathogen (Garitano et al., 2011; Pichel et al., 2012; Guzman-Herrador et al., 2011; Pichel and Caffer, 2016; Bakhshi et al., 2017). Another alternative method in the identification of isolates of food pathogens such as *Shigella* are those based on the analysis of proteins (mainly 2-20 KDa ribosomes) expressed by the genome through mass spectrometry, where a spectrum of specific masses, the “Footprint”, for each genus and species by using Matrix Assisted Laser Desorption Ionization - Time Of Flight - Mass Spectrometry (MALDI-TOF MS) (Bou et al., 2011; Schaumann et al., 2013; Paauw et al., 2015; Ragupathi et al., 2018; Huertas-Caro et al., 2019); those are among the characteristics of these systems in their use that they do not present extraction procedures. A bacterial colony can be used directly, demonstrating that it is possible to compare the spectra generated with previous databases, and also shows a speed of execution of the technique, obtaining and precision of results (Bou et al., 2011; Paauw et al., 2015).

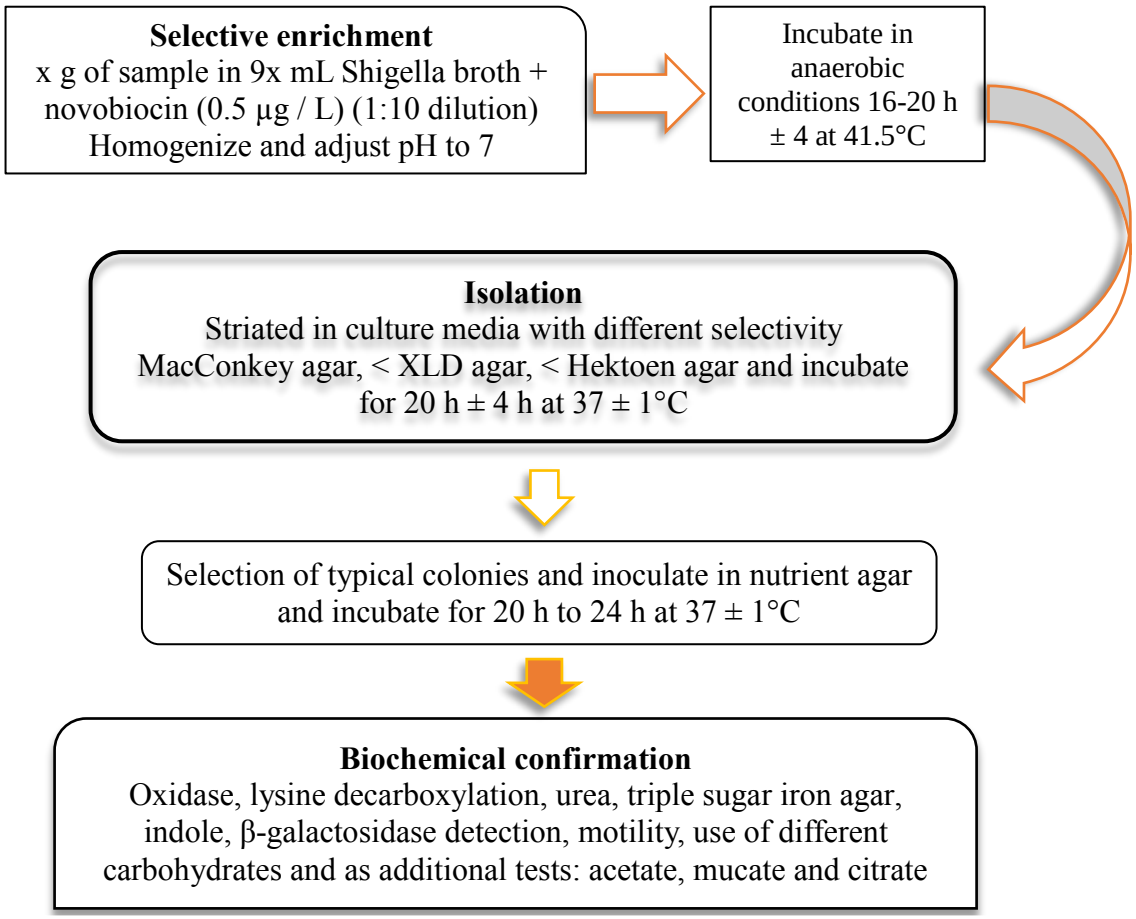

Figure 2. Flow chart for the isolation of *Shigella* spp., in food (ISO 21567:2004; ANMAT-RENAPRA, 2013).

CONCLUSIONS

Fish is one of the constituent foods of the human diet due to its nutritional value. Tilapia is among the world's most widely produced fish through fishing and aquaculture, mainly intended for human consumption. Fish, despite being a food of high nutritional value and part of the human diet, is very susceptible to deterioration and contamination through the different phases of the food chain by various bacterial agents that cause diseases, so it can be considered a sanitary risk food.

Shigellosis is a Foodborne disease caused by different species of bacteria of the *Shigella* genus. Foods related to disease cases and outbreaks are those eaten raw or subjected to inadequate cooking and storage conditions. The causative agents of shigellosis are cosmopolitan in distribution with humans and animals as a reservoir and can contaminate food, such as fish, through contamination with fecal material from culture or capture waters, as well as by inadequate hygiene conditions and practices during handling in production and processing. The general measures indicated to guarantee safety throughout the fish food chain and protect public health from shigellosis causative agents are the implementation of good hygiene practices and process controls, implementation of Hazard Analysis and Critical Control Points systems (HACCP), application of microbiological tests and their correlation with microbiological criteria for the monitoring of product quality and production conditions, as well as constant training in hygiene for food handlers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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