

Review

Food Safety and Fish Production the Case of *Staphylococcus aureus*: A Review

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Abstract: Currently food safety must be a mandatory condition in food available for human and animal consumption. Food safety is considered the characteristic that these will not cause harm to the health of consumers. Foodborne diseases are considered a serious public health problem throughout the world due to their incidence, mortality and negative repercussions on the economic and productive sectors. Among the most frequent cases and outbreaks of foodborne illness is food poisoning resulting from the consumption of foods such as fish and products contaminated by *Staphylococcus aureus* and toxins. *Staphylococcus aureus* contamination of food can occur at any stage of the food chain, from primary production, processing, distribution, marketing, even manipulation by the final consumer; this mainly due to the absence or inadequate hygiene conditions and practices, establishing that this pathogen has the human being as its main reservoir and source of contamination. Therefore, the purpose of this manuscript is to provide general information through the search and analysis of information in different academic and scientific databases on foodborne diseases, causal agents of these diseases, especially *Staphylococcus aureus*, related foods in its transmission such as fish and tilapia, sources and conditions of contamination, as well as prevention and control actions in the different phases of the food chain in favor of food safety and public health.

Keywords: Aquaculture, Fishing, Food Pathogens, Foodborne Diseases

Introduction

The safety of a food is the guarantee that it will not cause harm or disease to the person who consumes it (De la Fuente Salcido and Corona, 2010; González-Muñoz and Palomino-Camargo, 2012; Jorquera *et al.*, 2015). Also, it is an essential part of the quality characteristics of food, along with the nutritional, sensory and commercial ones (De la Fuente Salcido and Corona, 2010; González-Muñoz and Palomino-Camargo, 2012).

Foodborne diseases are considered a serious health problem worldwide due to their incidence, mortality,

the appearance of new forms of transmission, vulnerable population groups, increased resistance of pathogenic microorganisms to antimicrobials and socioeconomic impact (González Flores and Rojas Herrera, 2005; Olea *et al.*, 2012; Soto Varela *et al.*, 2016; WHO, 2020a). According to estimates from the World Health Organization (WHO), one in 10 people worldwide falls ill and 420,000 die a year, especially children (WHO, 2020b). These diseases are identified by presenting various symptoms in the gastrointestinal system, such as: Nausea, vomiting, diarrhea, abdominal pain and fever; presenting in severe cases complications such as sepsis, meningitis,

abortions, Reiter's syndrome, Guillan Barré syndrome, even death; and where the most vulnerable population groups to these diseases are children, the elderly, pregnant women, people with poor immune systems and those with high levels of poverty and poor hygiene conditions (Torrens *et al.*, 2015; Soto Varela *et al.*, 2016).

These diseases are derived from the consumption of food contaminated by more than 250 causal agents that can have a physical, chemical and/or biological origin (De la Fuente Salcido and Corona, 2010; Barreto Argilagos *et al.*, 2010; Olea *et al.*, 2012; Torrens *et al.*, 2015; Soto Varela *et al.*, 2016). This contamination can be generated at any stage of the food chain (from farm to table), thus losing its safety (De la Fuente Salcido and Corona, 2010; WHO, 2020a). Among the causal agents of biological origin are bacteria such as *Salmonella* spp., *Vibrio* spp., *Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., *Clostridium* spp., *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*, commonly related to cases and disease outbreaks (De la Fuente Salcido and Corona, 2010; Barreto Argilagos *et al.*, 2010; Olea *et al.*, 2012; Jorquera *et al.*, 2015; Torrens *et al.*, 2015; Soto Varela *et al.*, 2016). It has been established that among the factors that have contributed to the increase in the incidence of foodborne diseases are globalization in the food supply, changes in food production systems (minimal processing), emergence of antimicrobial resistant pathogens, along with the acquisition of virulence factors, climate change, demographic changes, changes in the lifestyle and eating habits of society such as the consumption of packaged foods, meals outside the home, the sale of prepared meals and fast foods (Olea *et al.*, 2012; Jorquera *et al.*, 2015; Torrens *et al.*, 2015; Vázquez-Sánchez *et al.*, 2018).

Foodborne diseases caused by bacteria can be: (A) Foodborne infections originating with the consumption of a food contaminated by the pathogen that is established and multiplies in the consumer and that has two variants: (1) Invasive infections: Characterized by the microorganism colonizing host's tissues and organs such as bacteria of the genus *Salmonella* spp., *Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Vibrio* spp., *Yersinia* spp. and entero-invasive *Escherichia coli*. (2) Toxin-infections: Those bacteria capable of colonizing and multiplying in the host's intestinal tract, where they excrete their toxins, such as: *Vibrio cholerae*, *Bacillus cereus* (enterotoxin-producing strains), *Clostridium botulinum*, *Clostridium perfringens* and enterotoxin-producing *Escherichia coli* or verotoxins; and (B) Food poisoning derived from toxins produced by bacteria that grew to a certain concentration in the food. Some microorganisms are: *Clostridium botulinum*, *Bacillus cereus* (producers of emetic toxin) and *Staphylococcus aureus* (Torrens *et al.*, 2015).

The appearance of these diseases is considered an indicator of the hygienic-sanitary quality of food, where the contamination of food can occur in its processing, or use of contaminated raw materials where various bacteria pathogenic to humans can be a constituent part of the microbiota of animals destined for consumption (González Flores and Rojas Herrera, 2005).

The purpose of this article is to provide a general description of foodborne diseases, causal agents of these diseases such as *Staphylococcus aureus*, related foods such as fish and tilapia, sources of contamination, as well as prevention measures and control in the different phases of the food chain in favor of food safety and public health.

Fish

Fish is all food extracted from fresh or brackish waters intended for human or animal consumption (Soares and Goncalves, 2012). The source of fish for humans is through capture fisheries and aquaculture activities where, according to estimates by the United Nations Food and Agriculture Organization (FAO) for the year 2018, the total world production was 178.5 million tons in live weight and a per capita consumption of 20.5 kg (FAO/WHO, 2020). Fish is considered a food source of protein with high digestibility and biological value, as well as unsaturated lipids, vitamins and minerals (Sousa *et al.*, 2011; Soares and Goncalves, 2012; Fuertes Vicente *et al.*, 2014; Ali, 2014). However, it is also a highly susceptible to contamination and deterioration food due to factors such as its pH close to neutrality, water activity and high nutrient content (Sousa *et al.*, 2011; Soares and Goncalves, 2012; Ali, 2014).

Among the factors involved in the deterioration of fish are the autolytic, chemical (oxidation) and microbiological activity, thus affecting its quality and safety (Huss, 1998; Soares and Goncalves, 2012; Fuertes Vicente *et al.*, 2014). The nutritional quality, freshness and safety of the fish vary depending on factors such as the species, age, conditions of the environment in which they live, type of feeding, conditions of capture, handling, conservation, transport and distribution (Fuertes Vicente *et al.*, 2014). Microbial activity plays a primary role in the deterioration and safety of fish and products due to numerous microorganisms present in the water, as well as the natural microbiota (Sousa *et al.*, 2011). The microbiota of fish is found on all external surfaces (skin and gills) and intestines, where the total number of microorganisms varies enormously and is highly dependent on the capture environment and species (Huss, 1997; 1998; Sousa *et al.*, 2011).

The microorganisms that are part of the fish microbiota represent an interest in terms of quality and

safety, since various genera and species of bacteria can cause human diseases. The bacterial microbiota in fish is made up of two groups: 1. Native species widely distributed in aquatic environments around the world, where water temperature clearly has a selective effect. Among these groups are *Clostridium botulinum*, *Listeria monocytogenes*, *Vibrio* spp., *Aeromonas* spp. and *Plesiomonas* spp. 2. Various *Enterobacteriaceae* non-native bacteria such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* and *Staphylococcus aureus* as a product of contamination of the aquatic environment they come from and due to poor production and handling conditions in the matter of hygiene (Huss, 1997; Romero-Jarero and Negrete-Redondo, 2011; Ali, 2014).

In fish, the microbial load depends, to a large extent, on the microbiological contamination of the aquatic environments, as well as the hygienic-sanitary level during the handling in cultivation, harvest, processing, storage and transportation practices (Alerte *et al.*, 2012; Fuentes *et al.*, 2011; Fuentes Vicente *et al.*, 2014). Aquaculture and fishing activities are widely distributed and provided with food, which has been related to outbreaks and cases of diseases; therefore, fish are considered potential carriers and transmitters of pathogens to humans, being the most relevant *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria* spp., *Aeromonas* spp. and *Vibrio* spp., (Romero-Jarero and Negrete-Redondo, 2011; Alerta *et al.*, 2012; Fuentes *et al.*, 2011; Espinosa *et al.*, 2015; Vázquez-Sánchez *et al.*, 2018; Ampuero *et al.*, 2018; Bujamma and Padmavathi, 2015).

Tilapia

Tilapias are freshwater fish of African origin that belong to the *Cichlidae* family and are classified into three genera based on their parental care patterns: *Tilapia* spp., *Oreochromis* spp. and *Sarotherodon* spp. (Baltazar, 2007; Jácome *et al.*, 2019). The species of the genus *Oreochromis* (*O. niloticus*, *O. aureus*, *O. mossambicus*) and their interspecific hybrids (red tilapia) have been considered as aquaculture organisms due to their ability to tolerate high densities, have a fast growing rate, resistant to diseases, easily adaptable to captivity, balanced diet and their meat is a source of quality protein and affordable price as a complete product or processed as fresh or frozen fillets, becoming an important food product of aquaculture with greater production and commercialization (Morales *et al.*, 2004; Vázquez-Sánchez *et al.*, 2018; Jácome *et al.*, 2019), being reported in 2018 a global aquaculture production within the finfish of 10.2% (FAO/WHO, 2020).

Tilapia is considered a nutritious food for humans, a source mainly of proteins (20.08 g/100 g), lipids (1.7 g/100 g), vitamin D (3.1 mg/100 g), B3 (3.9 mg/100 g), B9 (24 mg/100 g) and minerals as Na (52 mg/100 g), K (302

mg/100 g) and P (170 mg/100 g) (SAGARPA, 2018). Globally, China is the main producer, followed by Bangladesh, Brazil, Philippines and Mexico (SAGARPA, 2018). Eight different species are produced in Mexico: (1) tilapia *rendalli*, (2) *Oreochromis niloticus*, (3) *Oreochromis niloticus* variety *Stirling*, (4) *Oreochromis niloticus* variety *Rocky Mountain*, (5) *Oreochromis aureus*, *Oreochromis mossambicus*, (6) *Oreochromis mossambicus* variety *orange*, (7) *Oreochromis urolepis hornorum* and (8) *Oreochromis* sp. (*Oreochromis mossambicus* x *Oreochromis urolepis hornorum*) being the main producing states of the country: Chiapas, Jalisco, Guerrero, Michoacán, Morelos, Nayarit, Estado De Mexico, Puebla, Veracruz, Sinaloa, Zacatecas and Tabasco (INP, 2018; SAGARPA, 2018). Within food production activities such as fishing, aquaculture, processing and conservation of tilapia and products, there is a risk of microbiological contamination involving a risk to safety and public health; the extensive variation of microorganisms in tilapia may be due to geographical particularities, environmental conditions, depth and existing microorganisms in the water it comes from and hygienic handling and processing conditions. Some microbial genera especially linked to products are: *Aeromonas* spp., *Micrococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Plesiomonas* spp., *Listeria* spp., *Clostridium* spp., *Lactobacillus* spp., *Helicobacter pylori*, *Moraxellaceae* and *Enterobacteriaceae* (*Shigella* spp., *Salmonella* spp., *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Edwardsiella* spp., *Morganella* spp., among others), for which it becomes essential to identify points of contamination, prevalence of microbial species and consider strategies to avoid the dissemination of microorganisms throughout the food chain in order to guarantee the quality and final safety of products (Morales *et al.*, 2004; Boari *et al.*, 2008; Atyah *et al.*, 2010; Shinkafi and Ukwaja, 2010; Junior *et al.*, 2014; Fuentes *et al.*, 2011; Echandi and Ulate, 2012; da Rocha *et al.*, 2013; Abdel-Moein *et al.*, 2015; Kang *et al.*, 2017; Vázquez-Sánchez *et al.*, 2018; Sheyin and Solomon, 2017; Lerma-Fierro *et al.*, 2020).

Staphylococcus aureus

Staphylococci are organisms of universal distribution located in water, air, soil, wastewater, food, as well as surfaces and all articles that come into contact with humans and animals, where the nose has been established as its main reservoir, along with the throat, skin and intestinal tract and estimating that the rate of human carriers can be from 30 to 60% of healthy individuals (Huss, 1997; Acha and Szyfres, 2001; Cortés Sánchez *et al.*, 2017).

Members of the genus *Staphylococcus* are considered Gram positive bacteria in the form of cocci, grouped in clusters with a diameter between 0.5

and 1.5 µm, facultative anaerobes, catalase positive, no capsules producers, not sporulated, not motile, with a guanine-cytosine ratio (G + C) from 30 to 39% (Acha and Szyfres, 2001; Zendejas-Manzo *et al.*, 2014; Cortés Sánchez *et al.*, 2017). This genus has been reported to have 32 species, 16 of which are considered opportunistic microorganisms (Cortés Sánchez *et al.*, 2017). The most important species from a clinical and food safety perspective, since they are generators of infectious and intoxications, are: *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Staphylococcus intermedius*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus hominis* subsp. *hominis*, *Staphylococcus warneri*, *Staphylococcus simulans*, *Staphylococcus capitis* subsp. *ureolyticus*, *Staphylococcus auricularis*, *Staphylococcus cohnii* subsp. *Urealyticum* (Cortés Sánchez *et al.*, 2017).

Staphylococcus aureus is a pathogen that presents several virulence factors that in combination with the decrease in host's defenses, lead to colonization and diseases. Some of these factors are cytotoxins (alpha, beta, gamma and delta), nucleases, proteases, lipases, hyaluronidase, catalase, coagulase, collagenase, leucocidin; different Toxins Such as Toxic Shock syndrome (TSST-1), enterotoxins (SE) and exfoliative toxins, in addition to structural components such as peptidoglycan, protein A, adhesion factors, teichoic acids, capsular polysaccharides and the generation of biofilms (Cervantes-García *et al.*, 2014a; Zendejas-Manzo *et al.*, 2014; Cortés Sánchez *et al.*, 2017). *Staphylococcus aureus* gives rise to infections and conditions such as osteomyelitis, septic arthritis, abscesses, pneumonia, empyema, endocarditis, pericarditis, meningitis and diseases mediated by its toxins including food poisoning, scarlet fever, scalded skin syndrome and toxic shock syndrome (Arteaga Bonilla and Arteaga Michel, 2005).

Staphylococcus aureus is the etiological agent of one of the most frequent diseases caused by the consumption of contaminated food, such as food poisoning (Brizzio *et al.*, 2011; Macori *et al.*, 2016). Common symptoms can appear between 2 and 4 h after consuming contaminated food, those are nausea, vomiting, abdominal spasm, diarrhea and hyperthermia. Symptoms typically last 24 to 72 h with a tendency to recovery, but in severe cases, dehydration can lead to hypotension, shock and collapse (Huss, 1997; Acha and Szyfres, 2001; Jorda *et al.*, 2012). Poisoning generally originates from the consumption of foods with mainly protein content, subjected to poor hygiene practices in handling and storage, where the growth of *Staphylococcus aureus* is greater than 5 Log Colony-Forming Units (CFU)/g and enterotoxins are present (Acha and Szyfres, 2001; Jorda *et al.*, 2012; Macori *et al.*, 2016; Vázquez-Sánchez *et al.*, 2018; Elika, 2020). Raw and ready-to-eat foods such as dairy, fish, chicken,

eggs, meats and vegetables are usually the most related to food poisoning by this pathogen (Huss, 1997; Cortés Sánchez *et al.*, 2017; Elika, 2020).

The enterotoxins of *Staphylococcus aureus* (SE) do not affect the sensory characteristics of contaminated food, they are water-soluble compounds, low molecular weight, resistant to proteolytic and thermostable enzymes synthesized in the growth phase after the exponential, encoded in mobile genetic elements, such as prophages, plasmids and islands of pathogenicity and genomic islands (Huss, 1997; Brizzio *et al.*, 2011; Jorda *et al.*, 2012; Zendejas-Manzo *et al.*, 2014; Cortés Sánchez *et al.*, 2017; Vázquez-Sánchez *et al.*, 2018; Wakabayashi *et al.*, 2018; Elika, 2020). Their genetic expression is controlled by at least three regulation systems: Agr, sar and, a catabolic repression system, in addition to being influenced by factors such as the type of nutrients, substrate pH, temperature, atmosphere, NaCl and competing microorganisms (Huss, 1997; Brizzio *et al.*, 2011; Jorda *et al.*, 2012; Zendejas-Manzo *et al.*, 2014; Cortés Sánchez *et al.*, 2017; Vázquez-Sánchez *et al.*, 2018; Elika, 2020). Five types of enterotoxins (SE) are known (A, B, C, D and E), which are the most frequent in food poisoning, with A being the most prevalent in outbreaks (Acha and Szyfres, 2001; Cortés Sánchez *et al.*, 2017; Wakabayashi *et al.*, 2018) and where it has been estimated that the dose of toxins to produce disease is 1 to 5 µg/g food (González Ayala and Cecchini, 2020). In addition to the fact that coagulase positive *Staphylococcus aureus* strains are those that are mainly related to disease outbreaks, because very few coagulase negative strains are capable of producing enterotoxins (Acha and Szyfres, 2001; Zendejas-Manzo *et al.*, 2014; Macori *et al.*, 2016).

Staphylococcus aureus is a mesophilic microorganism with a minimum development temperature of 10°C, facultative anaerobic, tolerance to NaCl (7.5%) and capable of developing water activities as low as 0.86 and a minimum pH of 4.5 for development (Huss, 1997; Cervantes-García *et al.*, 2014a). *Staphylococci* are poor competitors and do not thrive in the presence of other organisms, therefore the presence of *Staphylococci* in naturally contaminated raw foods can be considered of limited relevance. However, in precooked foods contaminated with *Staphylococcus aureus* and under suitable conditions of time and temperature, rapid development and production of toxins can occur (Huss, 1997).

Staphylococcal contamination of food is generally related to an absent or poor hygiene practice in any of the phases of the food chain, being humans part of the ecological niche; contamination is mainly decanted in direct contact phases by food handlers in processing (Jorda *et al.*, 2012; da Rocha *et al.*, 2013; Vázquez-Sánchez *et al.*, 2018; Cortés Sánchez *et al.*, 2017). Furthermore, *Staphylococcus aureus* can form biofilms on food contact surfaces that

increase its tolerance to stress conditions, as well as its persistence and contamination in environments related to food generation (Vázquez-Sánchez *et al.*, 2018).

Actions to Control Food Safety and Prevention of Diseases Caused by *Staphylococcus aureus*

The responsibility for food safety is shared as it involves the government health authorities as well as those who produce process and market them and it is an obligation to ensure they are safe. However, there may be oversights or omissions in this regard, which are reflected in a risk and a damage to the consumers' health (González Flores and Rojas Herrera, 2005; González-Muñoz and Palomino-Camargo, 2012). Fish can be contaminated at any stage of the production chain from primary production, handling after capture, processing, conservation and storage, being determinants for the quality of the final product (Farias and Freitas, 2008; Sousa *et al.*, 2011; Buijamma and Padmavathi, 2015; Vázquez-Sánchez *et al.*, 2018). Therefore, the sanitary regulation establishes different microbiological indicators and limits to the presence in food to guarantee safety and quality (Farias and Freitas, 2008; Vázquez-Sánchez *et al.*, 2018). There are different microorganisms that indicate contamination and sanitary risk, such as *Staphylococcus aureus*, whose study is of relevance for the surveillance of the hygienic quality of fishery products due to its relationship in the generation of diseases through food (Romero-Jarero and Negrete-Redondo, 2011).

Fish have a microbiota that is dependent on what it exists in the waters where they live, meaning that polluted waters can provide a variety of pathogens; the microorganisms in the skin and gastrointestinal content of the fish in life do not invade the sterile muscle package due to the protection of its natural defenses. When the animal dies, these microorganisms penetrate the fish, causing deterioration and loss of safety. The presence of *Staphylococcus aureus* in fish and products can mainly indicate contamination from the skin, mouth and nostrils of infected handlers or healthy carriers, during stages of capture, transport, storage, processing and preparation processes (Huss, 1997; Romero-Jarero and Negrete-Redondo, 2011).

In order to guarantee safety in the production of fish and products, the need for the implementation of good hygiene practices and conditions, Sanitation Standard Operating Procedures (SSOP), as well as Hazard Analysis and Critical Control Points (HACCP) systems has been established in the different phases of the food chain (aquaculture, fishing, handling, processing, elaboration and preservation of products) which include compliance with microbiological

criteria of raw materials susceptible to contamination by *Staphylococcus aureus*, the control of temperature maintaining the cold chain (<6°C) during transport, storage and distribution of food to avoid microbial development or keep food with pH <5, in addition to the control of health and hygiene of the personnel that handles and elaborates food in order to avoid contamination, proliferation and production of staphylococcal toxins in fishery products (Huss, 1997; Acha and Szyfres, 2001; CXC/RCP, 1969; OMS, 2007; Fitzsimmons, 2008; Kopper *et al.*, 2009; OMS, 2016; Goulding, 2016; Gutiérrez-Guzmán *et al.*, 2017; Elika, 2020).

In regards of control and prevention of food poisoning by *Staphylococcus aureus* through fish, different sanitary regulations have been established around the world. In Latin American, countries such as Mexico, through the National Service of Health, Safety and Food Quality (SENASICA acronym in Spanish) has published the manual for the implementation of good aquaculture practices in primary fish production including those for tilapia farming (SENASICA, 2019), as well as the official standard "(NOM-242-SSA1-2009)", within its sanitary regulation, establishes the microbiological criteria (*Staphylococcus aureus* and toxins) for fishery products, with a maximum limit of 1000 CFU/g of product and absence of toxins and "(NOM-251-SSA1-2009)" establishes the requirements for good hygiene practices for the food process and specifies the principles for the implementation of the HACCP system. Finally, the standard "(NMX-F, 2016)" involves the hygienic handling in the service of food prepared in fixed establishments and so they can obtain the Distinctive "H" recognition the Ministry of Tourism and the Ministry of Health provide to those establishments that have met the hygiene standards.

In South America, countries such as Peru, ministerial resolution "(SA/DM, 2003)" establishes the microbiological criteria for food and beverages for human consumption in the national market must comply, where for chilled or frozen raw hydro biological products (Fish) the microbiological criteria for *Staphylococcus aureus* the maximum allowable limit is 10³ CFU/g. While in Central America through the Central American Technical Regulation of food safety as "(RTCA 67.04.50:17. ICS 67.050, 2018)" establishes the microbiological criteria for *Staphylococcus aureus* in raw products, such as frozen or refrigerated fish, the permitted limit of 10² CFU/g of product for commercialization and consumption in the territory of countries in the region such as Guatemala, Salvador, Costa Rica, Nicaragua, Honduras and Panama.

In the European Union, food safety is established in different regulations throughout the food production

chain. Some of these regulations are regulation (CE) No 178/2002 (OJEU, 2002), which establishes the principles and general requirements of the food legislation and the European Food Safety Authority is created and procedures related to food safety are established. While regulation (EC) No 852/2004 (OJEU, 2004a) establishes general rules for food, business operators in the matter of hygiene of food products, that includes the general application of procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP). Meanwhile, regulation (EC) No 853/2004 (OJEU, 2004b) establishes specific rules for food business operators regarding the hygiene of food of animal origin and the regulation (CE) No 854/2004 (OJEU, 2004c) established for the organization of official controls of the products of animal origin destined for human consumption. Regulation (CE) No 882/2004 (OJEU 2004d) on official controls carried out to guarantee the verification of compliance with the legislation on feed and food and the regulations on animal health and animal welfare. Directive 96/23 CE (Directive, 1996) that refers to the control measures applicable to certain substances and their residues in live animals and their products, including aquaculture and regulation (EC) No 2073/2005 (OJEU, 2005), which establishes microbiological criteria applicable to food, including fishery products for their acceptability, safety and marketing.

Laboratory Analysis

Regarding the control and prevention of food poisoning by *Staphylococcus aureus*, the detection and isolation of the pathogen in food is an essential part of the process; for that, various analytical methodologies have been developed around the world, some of them have standardized that involve isolation and enumeration through the use of selective and differential culture media (Baird-Parker, mannitol salt agar, Staphylococcal agar No. 110, DNase agar), as well as biochemical identification tests (catalase, coagulase and thermonuclease) for colonies with characteristic or typical growth (Zendejas-Manzo *et al.*, 2014) (Table 1). Among the standard developed methods are that of (Tallent *et al.*, 2019) in the U.S. Food and Drug Administration (FDA) through the Bacteriological Analytical Manual (BAM), where the method involves isolation and enumeration of *Staphylococcus aureus*, generating dilutions of the sample with phosphate-saline buffer and 1% NaCl. 1 mL of the dilution is distributed on three plates of Baird Parker agar (0.4, 0.3 and 0.3 mL) and incubated for 45-48 h at 35-37°C. Colonies of typical growth are selected (circular, smooth, convex,

moist, 2-3 mm in diameter, gray to black in color, surrounded by an opaque area and frequently with a light outer area of buttery to rubbery consistency) and confirmation from biochemical tests (coagulase, catalase, anaerobic utilization of glucose and mannitol and thermonuclease). Likewise, the method of the Most Probable Number (MPN) recommended for the regular surveillance of foods in which small amounts of *Staphylococcus aureus* and/or population of competing species are expected is established. For which, using 3 tubes with trypticase soy broth with 10% NaCl and 1% sodium pyruvate, they are inoculated with 1 mL of the diluted sample and incubated at 48±2 h at 35-37°C. Baird-parker agar is inoculated from each tube and incubated for 48 h at 35-37°C. The growing colonies are subjected to biochemical confirmation tests (coagulase, catalase, anaerobic use of glucose and mannitol and thermonuclease).

For their part, the United States Department of Agriculture (USDA) and the Food Safety and Inspection Service (FSIS) are assisted in their regulatory activities with The Microbiology Laboratory Guidebook (MLG) that contains test methods for the isolation and identification of different food pathogens such as *Staphylococcus aureus* through quantitative analysis as a sanitary indicator in fish and other foods (MLG 3.02). The MLG 3.02 method involves a direct plate count for *Staphylococcus aureus* through dilution and homogenization of the sample and inoculation in differential and selective culture medium such as Baird-Parker, as well as the confirmatory biochemical test (coagulase activity) for colonies with typical growth (Fig. 1) (USDA, 2020).

Another widely used standard method is from International Organization for Standardization (ISO) ISO 6888 Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). ISO 6888-1:1999/AMD (2018) Technique using Baird-Parker medium, (ISO 6888-2:1999) Technique using rabbit plasma fibrinogen agar medium and (ISO 6888-3, 2003) Detection and Most Probable Number (MPN) technique for low numbers.

Meanwhile, in Latin America in Mexico, the official sanitary regulation through the official standard “(NOM-210-SSA1-2014; NMX-F-310-1978)” that establish the methods for the microbiological analysis of sanitary indicators and food pathogens such as *Staphylococcus aureus* in the food (Figs. 2 to 4) indicating that frozen fish samples subject to analysis must be thawed in refrigeration (2-5°C) a maximum of 18 h before analysis. On the other hand, in South America in countries like Peru, the Ministerial Resolution

“(MINSA, 2007)” establishes the technical guide for the hygiene analysis of surfaces in contact with food, including live surfaces (food handlers) focused on sanitary control. Likewise, alternative methods have

been developed for the detection of *Staphylococcus aureus* and toxins in food based on immunoassays (Suárez-Semour and González, 1990; Figueroa *et al.*, 2002; Brizzio *et al.*, 2013).

Table 1: Biochemical identification characteristics for *Staphylococcus aureus* isolates (NOM-210-SSA1-2014; Tallent *et al.*, 2019)

Test	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Micrococcus</i>
Catalase activity	+	+	+
Coagulase activity	+	-	-
Thermonuclease activity	+	-	-
Glucose fermentation	+	+	-
Mannitol fermentation	+	-	-

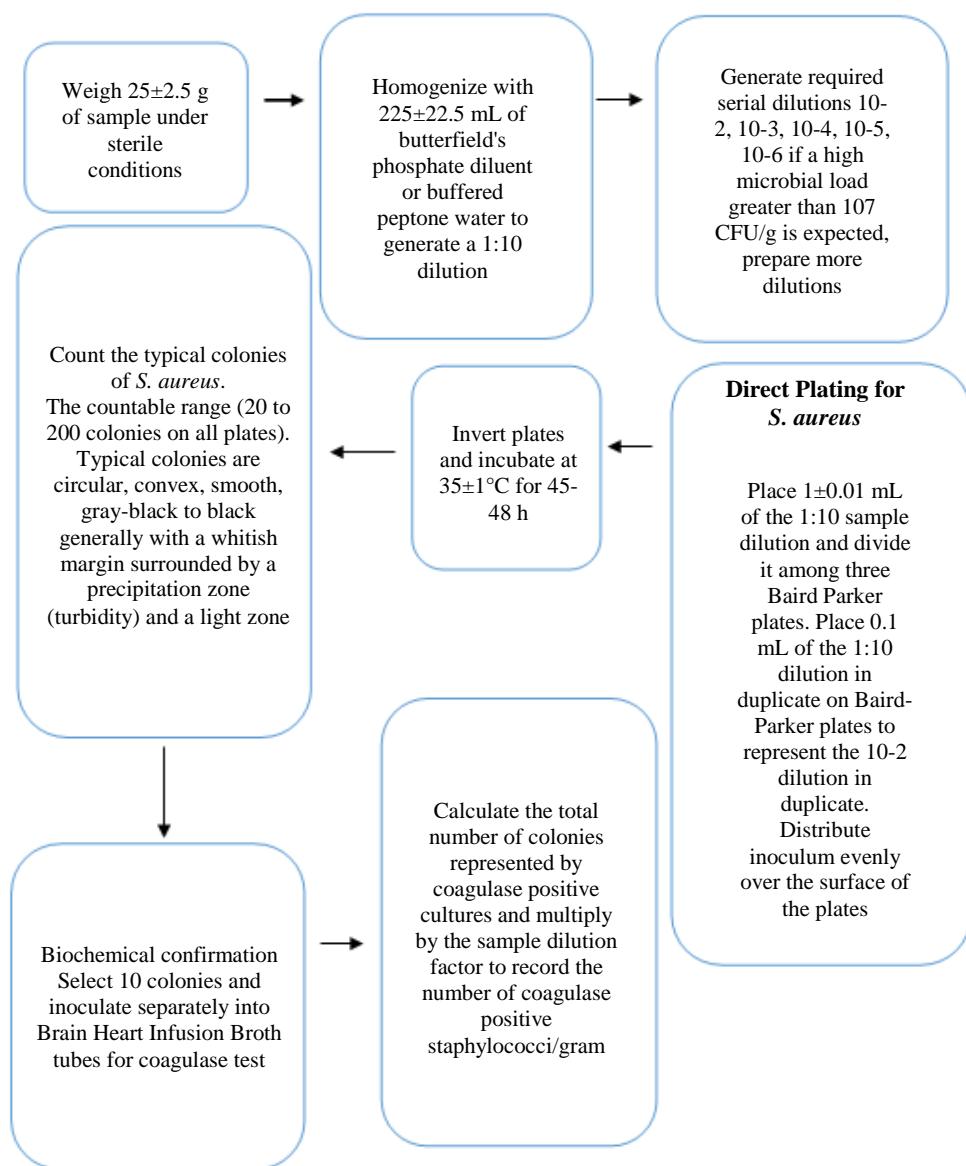


Fig. 1: Flow diagram for isolation and quantification of *Staphylococcus aureus* (MLG 3.02) (USDA, 2020)

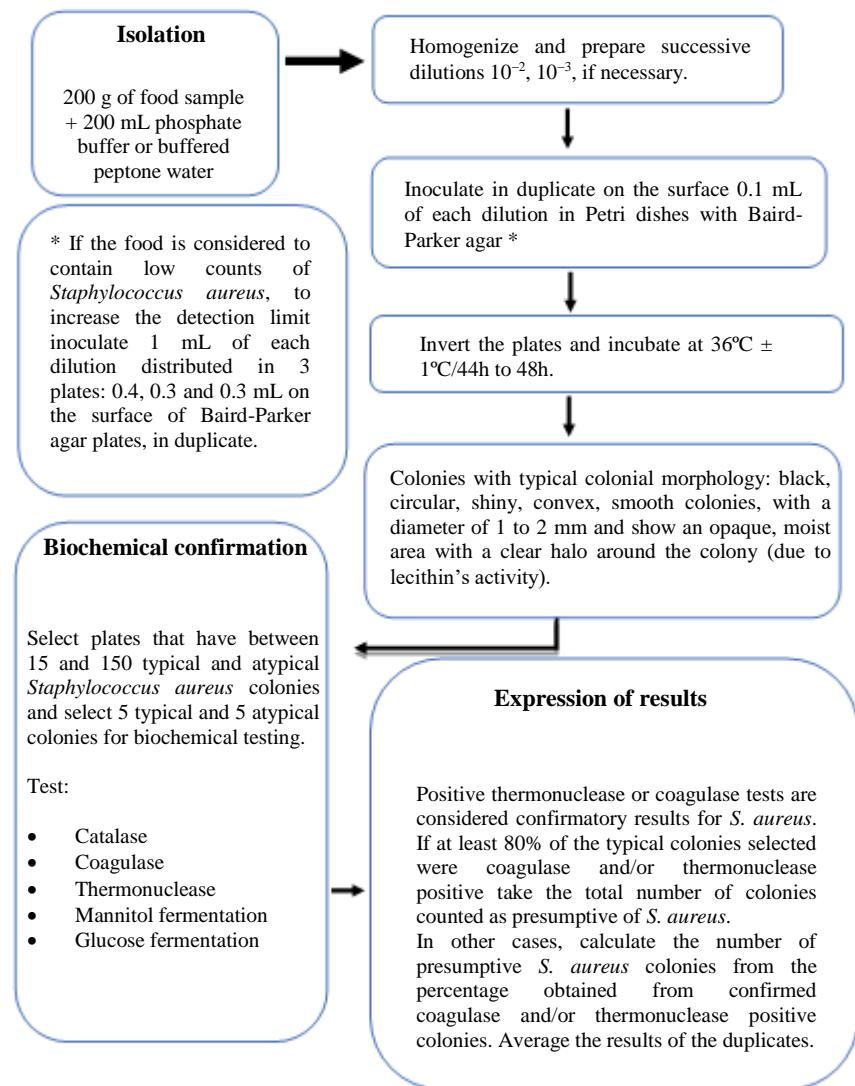


Fig. 2: Flow chart for the isolation and enumeration of *Staphylococcus aureus* in food (NOM-210-SSA1-2014)

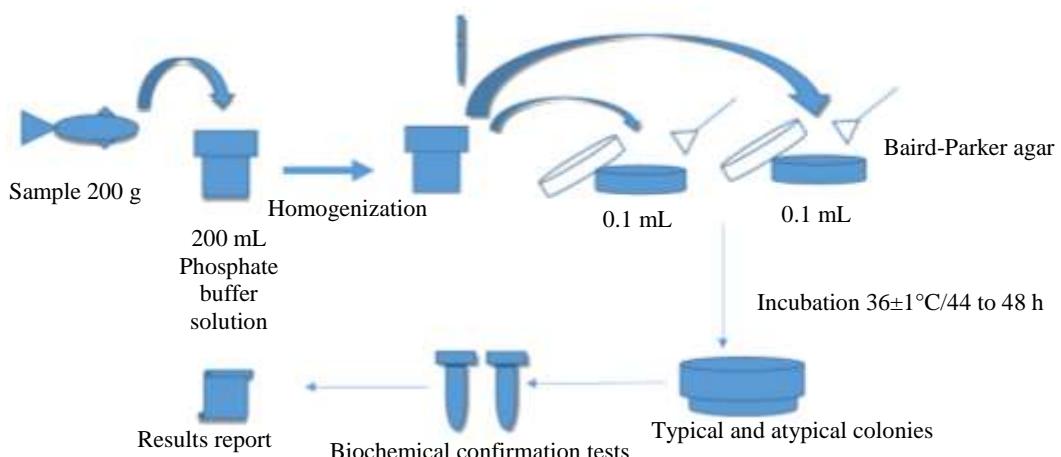


Fig. 3: Scheme of work for the isolation and enumeration of *Staphylococcus aureus* in fish (NOM-210-SSA1-2014)

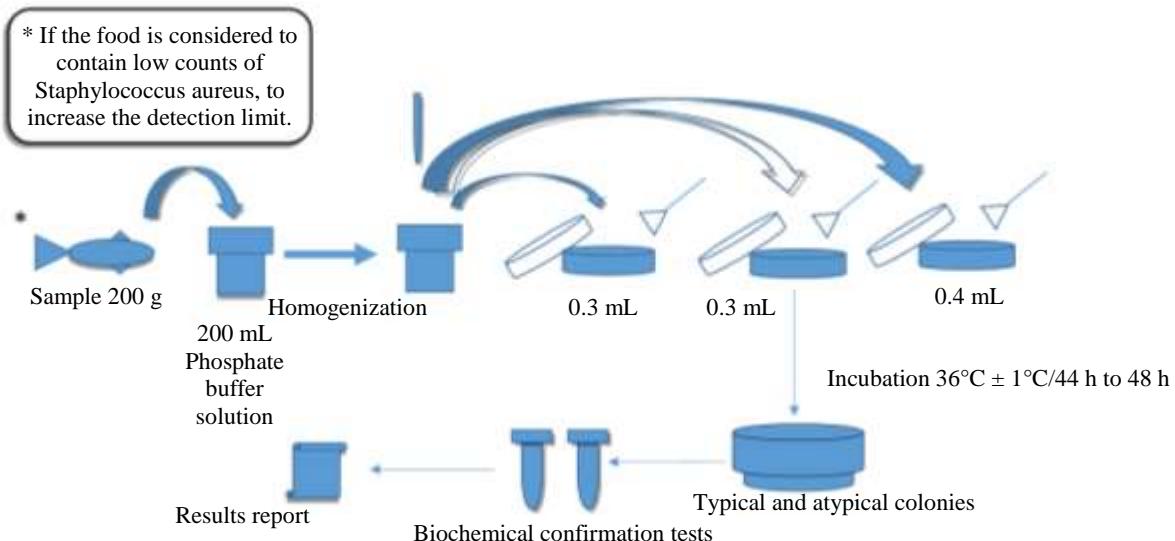


Fig. 4: Scheme of work for the isolation and enumeration of *Staphylococcus aureus* in fish (NOM-210-SSA1-2014)

Also, molecular methods based on the Polymerase Chain Reaction (PCR) in its different variants using the respective target genes encoding mainly enterotoxins (SE) (*sea*, *seb*, *sec*, *sed*, *see* and 16S rDNA) have been developed. These methods allow a greater number of samples to be analyzed, greater sensitivity, specificity and speed in obtaining results compared to traditional methods, they also allow epidemiological, evolutionary and phylogenetic studies (Manfredi *et al.*, 2010; Saito *et al.*, 2011; Jorda *et al.*, 2012; Brizzio *et al.*, 2013; Palomino-Camargo and González-Muñoz, 2014; Arfatahery *et al.*, 2016; Wakabayashi *et al.*, 2018; Hussein *et al.*, 2019).

Antimicrobial Resistance by *Staphylococcus aureus* and Food Safety

Antimicrobial resistance is defined as the ability of a microorganism to resist the inhibitory activity of its growth or death by a certain antimicrobial (Verraes *et al.*, 2013). Antimicrobial resistance has been considered a threat and a serious problem for human and animal health worldwide for several years (Puig Peña *et al.*, 2019; FAO/WHO, 2020).

The increase in resistant microorganisms, being mainly bacteria, impacts on public health due to the increase in morbidity and mortality rates due to infections, extension of periods of disease and increased health costs (Peña *et al.*, 2011). Resistance to antimicrobials by bacteria can be naturally acquired as a product of chromosomal mutations and exchange of genetic material with other bacteria or phages, by mechanisms of transformation, transduction, transposition and conjugation (Becerra *et al.*, 2009; Verraes *et al.*, 2013; Calderón Rojas and Aguilar Ulate, 2016). The different resistance mechanisms are: Modification of the

permeability of the membrane, extraction of the compound by efflux or expulsion mechanisms, enzymatic inhibition, modification of the attack target or alteration of the composition and content of the cell wall glycoproteins and formation of biofilms (Becerra *et al.*, 2009; Peña *et al.*, 2011; Verraes *et al.*, 2013; Gestal *et al.*, 2014; Calderón Rojas and Aguilar Ulate, 2016).

Antimicrobial resistance in bacteria has been considered to be derived from a synergy in the excessive and inappropriate use of antimicrobials in humans and animals; and in the latter, antimicrobials are used for different purposes such as therapeutic, prophylactic and growth promotion for food production (Puig Peña *et al.*, 2019; Verraes *et al.*, 2013). Antimicrobial resistance affects food safety as food plays an important role in the development and spread of antimicrobial resistance by microorganisms; in addition to being the production systems and food chains a potential route of exposure together with the environment and water (Puig Peña *et al.*, 2019; FAO/WHO, 2020).

So far and among the main foodborne pathogens that have been reported with antimicrobial resistance are: *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*, *Listeria* spp., *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp. and *Shigella* spp. (Junod *et al.*, 2013; Calderón Rojas and Aguilar Ulate, 2016; Elmahdi *et al.*, 2016; Cáceres *et al.*, 2017; Weiler *et al.*, 2017). In the case of *Staphylococcus aureus*, the isolation of strains resistant to various Antimicrobials Such as Resistant Methicillin (MRSA) has been reported, which has caused various infections both in the hospital environment and in the community; it has been considered of relevant negative impact on public health. Likewise, resistant strains have been isolated in food and food handlers, which has guided the

implementation of hygiene measures and epidemiological studies (Cerdeira and Almeida, 2013; Cervantes-García *et al.*, 2014b; Peña *et al.*, 2015; Macedo *et al.*, 2016).

In aquatic environments and food production systems such as aquaculture and because of the use of antibiotics, it allows both saprophytic and pathogenic microorganisms to exchange resistance genetic material, constituting that a risk to human health (Sáenz *et al.*, 2019; Puig Peña *et al.*, 2019). In various studies around the world, isolates of bacteria resistant to various antimicrobials, including *Staphylococcus aureus*, have been reported from fish and shellfish, reflecting the presence of this phenomenon and the health risk associated with these foods (Albuquerque *et al.*, 2007; Atyah *et al.*, 2010; Soliman *et al.*, 2014; Tiamiyu *et al.*, 2015; Boss *et al.*, 2016; Puig Peña *et al.*, 2019).

Among the actions to combat this phenomenon, to prevent its development and reduce its spread, international organizations, such as FAO, have established codes and guidelines (CAC/RCP, 61-2005; CAC/GL, 77-2011). Likewise, to control this phenomenon in different phases of the food chain, codes have been developed regarding the use of veterinary drugs and their residues (CX/MRL, 2-2018), animal feed (CAC/RCP 54-2004) and good practices of hygiene (CXC/RCP, 1-1969; CXC, 52-2003) to be applicable in the different phases of the food chain, the latter being considered fundamental for food safety and combating antimicrobial resistance (Peña *et al.*, 2011; Verraes *et al.*, 2013; OMS, 2016; FAO/WHO, 2020).

Conclusion

Fish is a highly nutritious food produced and marketed around the world. However, it is very susceptible to spoilage and contamination throughout the food chain. Tilapia are fish that constitute an important source of protein in the human diet, either as fillet or whole and it is one of the species with the highest production mainly through aquaculture worldwide.

Foodborne diseases constitute a serious public health problem at a global level and where foods such as fish are mainly related to outbreaks and cases of diseases, including food poisoning.

Staphylococcus aureus is an important microorganism in human clinical and food safety because, it is capable of presenting resistance to different antimicrobials and generating different diseases, including food poisoning through contamination and consumption of food, where it has growth and generation of toxins. As it is considered a pathogen of cosmopolitan distribution and the human being one of its main reservoirs around the world, different actions have been developed and established for the surveillance, control and prevention of food contamination in the different phases of the food chain, as well as for the fight against antimicrobial resistance, such as the

implementation of good hygiene practices, quality assurance systems (HACCP) and microbiological criteria for the acceptance of products for consumption with emphasis on the hygiene of food handlers and storage conditions.

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Author Contributions

Alejandro De Jesús Cortés-Sánchez: Conception and design of the study, search, information analysis, writing of the manuscript.

Mayra Díaz-Ramírez, Ma. De la Paz Salgado-Cruz and Rocío G. Hernandez-Nava: Search, information analysis, writing of the manuscript.

Interest Conflict

The authors declare that they have no conflict of interest in the development and publication of this document.

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