Yersiniosis and fish consumption

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ABSTRACT

Foodborne diseases are considered a serious public health problem worldwide due to their incidence and mortality. The increase in the appearance of those diseases with their negative effects is contributed to the changes in society's eating habits, globalization of the production, and food marketing. Food safety is currently a fundamental characteristic in the production and availability of food, being therefore indicated as the guarantee that will not harm the consumer's health. Fish is considered a nutritious and globally marketed food and its processing and consumption occur in different forms and presentations (whole, whole gutted, fillet, fermented, canned, salted, cooked, smoked, raw, among others). Fish, due to its chemical composition and the hygienic conditions to which it is subjected during its production, handling, processing and conservation, is highly susceptible to be contaminated and hence, deteriorated by various microorganisms. Some of these are food pathogens as *Yersinia enterocolitica*, which is identified as a causal agent of the zoonosis called yersiniosis that may put the health of consumers at risk. The objective of this review is to provide general information on foodborne diseases, especially those whose causative agent is *Yersinia enterocolitica* through the consumption of fish. Characteristics of the pathogen, aspects of control, and prevention of food contamination are presented, including microbiological analysis and sanitary regulation in different parts of the world. Additionally, reference is made to aspects of the phenomenon of antimicrobial resistance and the danger for public health.

INTRODUCTION

Foodborne Disease (FD) is the syndrome caused by the consumption of food and / or water contaminated with physical, chemical or biological agents negatively affecting the health of the consumer (**Torrens *et al*., 2015; Soto *et al*., 2016; WHO, 2019**). Food contamination can be generated during the production, processing, handling, conservation, transport or marketing, that is, at any stage of the food chain (from the farm to the consumer's table) (**Li, 2015; WHO, 2019**).

FD is generally characterized by gastrointestinal symptoms that involve nausea, vomiting, diarrhea, abdominal pain, and fever; in addition to presenting complications such as sepsis, meningitis, abortions, Reiter's syndrome, Guillan Barré syndrome, or death (**Soto** *et al.***, 2016; WHO, 2019**).

The FD caused by biological agents are classified into two types: the first tometion is the food infections that are derived when a pathogen, that establishes itself and multiplies in the consumer, is present in food and can be invasive when the microorganism colonizes tissues and organs of the host (*Salmonella* spp*., Aeromonas* spp*., Yersinia* spp., and *E. coli*). To illustrate, a pathogen can also be non-invasive capable of colonizing and multiplying in the intestinal tract of the host where it excretes toxins (*Vibrio cholerae* and *Clostridium perfringens*). Seconly: food poisoning as a result of the consumption of toxins synthesized by bacteria that developed up to a certain concentration in the food mediated by the quorum sensing mechanism (*Clostridium botulinum, Bacillus cereus and Staphylococcus aureus*) (**Esesarte, 2002; Torrens** *et al.***, 2015**).

Estimations from the World Health Organization (WHO) indicated that those diseases affect one in 10 people annually, causing 4,000,000 deaths worldwide, specifically in children under 5 years (**WHO, 2019**). The global incidence of FD may be due to various factors such as globalization and commercialization of food, new food production systems, increased demand for ready-to-eat foods, changes in demographic behavior and lifestyle of population, emergence of susceptible or vulnerable population groups, emergence of resistant microorganisms, and impact on terrestrial ecosystems (climate change) (**Lopez** *et al.***, 2013; Jorquera** *et al.***, 2015; Torrens** *et al., **2015**).

FD are considered a global public health problem, due to their incidence, mortality, economic costs in public health, animal and food industry; more than 250 causative agents of those diseases have been reported. Those of biological origin of bacterial type such as *Staphylococcus aureus, Escherichia coli, Salmonella* spp., *Vibrio* spp*., Campylobacter* spp*., Bacillus cereus, Listeria monocytogenes, Yersinia enterocolitica*, were determined among others (**Carrillo** *et al.***, 2011; Li, 2015; Jorquera** *et al., **2015 Torrens** *et al.***, 2015; Hernández** *et al., **2017; Cortés-Sánchez** *et al.***,2019; WHO, 2019**).

Moreover, Yersiniosis is often derived from the consumption of raw or undercooked pork meat contaminated with the *Yersinia enterocolitica* bacteria. It is estimated that this bacteria causes each year, in the United States of America only, about 117,000 diseases, 640 hospitalizations, and 35 deaths, with children being the most affected during winter (**CDC, 2016**). While in the European Union, in 2017, 6,823 cases of yersiniosis were reported in 26 member states, being the third most reported zoonosis only behind salmonellosis and campylobacteriosis where *Yersinia enterocolitica* was the mostly

related species in human cases, and the bioserotype detected was 4 / O: 3 followed by 2 / O: 9 and 2 / O: 5.27 (EFSA-ECDC, 2018).

Foods have various functions in living things such as the supply of energy and nutrients necessary to promote and sustain growth, maintain bodily functions, replace or repair tissues. However, in order to achieve these functions, it doesn't only require its availability, but also its safety when produced, handled, and consumed in a hygienic way being considered an important source of exposure to risks due to the presence of various chemical or biological agents that can compromise human health **(Lopez *et al*., 2013; Jorquera *et al*., 2015)**.

The objective of this review is to provide general information on foodborne diseases, especially those whose causative agent is *Yersinia enterocolitica* through the consumption of fish. The current study spotlighted the characteristics of the pathogen, aspects of control and prevention of food contamination, including microbiological analysis and sanitary regulation in different parts of the world. Furthemore, reference is made to aspects of the phenomenon of antimicrobial resistance and the danger for public health.

1. *Yersina* **spp.**
The genus *Yersinia* belongs to the *Enterobacteriaceae* family. It is composed of 18 species, *Y. aldovae, Y. aleksiciae, Y. bercovieri, Y. entomophaga, Y. frederiksenii, Y. intermedia, Y. kristensenii, Y. massiliensis, Y. mollaretii, Y. nurmii, Y. pekkanenii, Y. rohdei, Y. ruckeri, Y. similis, Y. wautersii, Y. enterocolitica, Y. pestis,* and *Y. pseudotuberculosis* **(Fredriksson & Laukkanen, 2018)**. The last three species are of a pathogenic nature and medical importance in generating disease to the human being called "yersiniosis" **(Romero, 2007; Weagant & Feng, 2007; Elika, 2013; Dekker & Frank, 2015; Aziz & Waheed, 2019)**. While *Y. ruckeri* is a fish pathogen generating large economic losses in the aquaculture sector **(Eissa *et al*., 2008; Carson & Wilson, 2009; Kumar *et al*., 2015)**.

The aforementioned bacteria have a cosmopolitan distribution and a cocobacillus form, gram-negative, 0.5 to 0.8 μm in diameter and 1 to 3 μm in length, mobile at 22 °C and immobile at 37 °C and capsule, but not spores **(Romero, 2007; Ramirez *et al*., 2015; Dekker & Frank, 2015)**. They have a genome of 4.0 to 4.9 Mb, mol% G + C is: 46–50, they are psychrotrophic microorganisms showing growth at temperatures of -1°C to 42°C, the optimum being 25°C to 32 °C. In addition, they can tolerate alkaline conditions, their growth pH is 4-10 and water activity is 0.95 to 0.99 **(Bottone *et al*., 2015; Dekker & Frank, 2015; Fredriksson & Laukkanen, 2018; Jimenez, 2018)**. Metabolically, they are aerobic and anaerobic facultative, lactose and oxidase negative, positive fermenters, catalase and urea positive; this was the last test of confirmation for

enteropathogenic species **(Romero, 2007; Ramirez *et al*., 2015; Dekker & Frank, 2015; Fredriksson & Laukkanen, 2018).**

Y. enterocolitica has been related to outbreaks of foodborne diseases being thus responsible for the food zoonosis called "yersiniosis", where the human being is an incidental host and is not part of the life cycle of the bacteria **(Weagant & Feng, 2007; Dekker & Frank, 2015; Jimenez, 2018; Aziz & Waheed, 2019).** This bacterium can be found in the environment and as part of the microbiota of the digestive tract of several animals (cats, dogs, sheep, goats, cattle, chickens, pigs, rodents, oysters, shrimp, rabbit, among others) but not of the human being; the route of transmission is fecal-oral through contaminated food and water such as meats (pig, goat, bovine, and sheep), oysters, vegetables, fish and raw milk, and is also able to survive and grow at temperatures of refrigeration **(Gonzalez & Quiñones, 2005; Romero, 2007; Weagant & Feng, 2007; Elika, 2013; Dekker & Frank, 2015; Gupta *et al.,* 2015; PAHO, 2018)**.

The infective dose of *Y. enterocolitica* is 1 x 10^9 CFU **(Gonzalez & Quiñones, 2005)** and the disease is characterized by presenting terminal ileitis, lymphadenitis, acute enterocolitis. Within the complications of the disease that occur are the rash, appendicitis, joint pain and bacteremia; symptoms usually appear 4–7 days after exposure and may last 1 to 3 weeks and may have post-infection sequelae such as erythema nodosum, arthritis, and glomerulonephritis **(Gonzalez & Quiñones, 2005; Dekker & Frank, 2015; Gupta *et al*., 2015; Younis et al., 2019)**. It is worthy to mention that the most susceptible population to risk of infection are children, elders, diabetics and immunosuppressed **(Elika, 2013; Dekker & Frank, 2015; Le Guern *et al*., 2016).**

Y. enterocolitica is divided into two subspecies based on the 16S rRNA gene sequence: subsp., enterocolitic that includes highly pathogenic strains, and subsp. palearctic including little pathogenic and non-pathogenic strains **(Fredriksson & Laukkanen, 2018)**. The *Y. enterocolitica* strains have a 4.6 Mb chromosome **(Ramirez *et al*., 2015),** their DNA exhibits 10-30% homology with respect to other genera of the *Enterobacteria* family **(Gupta *et al*., 2015)**. They are classified serologically into groups according to their thermostable somatic antigens **(Weagant & Feng, 2007; Sabina *et al*., 2011)**. Six biovars (1A, 1B, and 2–5) and more than 50 serogroups have been described for *Y. enterocolitica* establishing that the pathogenic strains belong to serogroups O: 1, 2a, 3; O: 2a, 3; O: 3; O: 8; O: 9; O: 4.32; O: 5.27; O: 12.25; O: 13a, 13b; O: 19; O: 20; and O: 21, predominantly in human diseases O: 3, O: 8, O: 9 and O: 5.27 **(Weagant & Feng, 2007; Ye *et al*., 2016).**

The virulence factors involved in the pathogenicity of *Y. enterocolitica* are located within the chromosome and in a 70 kb virulence plasmid (pYV) detectable only in

virulent strains whose products include YadA and Yops proteins **(Sabina *et al*., 2011; Fàbrega & Vila, 2012; Ye *et al*., 2016; Fredriksson & Laukkanen, 2018).**

Y. enterocolitica presents factors of adhesion to epithelial cells and invasion of intestinal mucosa through different proteins such as: YadA, Ail and invasive (Inv). On the other hand, the flagella contribute to the motility for the invasion of the host cell, in addition to presenting a thermo stable enterotoxin (Yst) associated with diarrheal processes **(Sabina *et al*., 2011; Fàbrega & Vila, 2012; Ye *et al*., 2016).**

The Yops and Ysc proteins are related to the interference and resistance to phagocytosis performed by macrophages and neutrophils of the host **(Sabina *et al*., 2011; Fàbrega & Vila, 2012).** The membrane lipopolysaccharides (LPS) intervene in the virulence of this pathogen through its constituents: (i) lipid A associated with toxicity; (ii) the core, consisting mainly of sugars; and (iii) the polysaccharide O antigen chain, associated with antigenic properties **(Fàbrega &Vila, 2012)**. Finally, *Yersinia* species also present yersiniabactin, a siderophic iron absorption system whose synthesis and regulation genes are found in mobile genetic elements and pathogenicity islands capable of transferring horizontally to other *Enterobacteria* species **(Dekker &Frank, 2015)**.

In addition, the importance of food safety and production with respect to pathogens such as *Y. enterocolitica* lies in its ability to generate biofilms **(Venegas *et al*., 2009; Novoslavskij *et al*., 2016; Younis *et al*., 2019).** The biofilms are formed from a community of microorganisms surrounded by a matrix of their own production consisting of an extracellular polymeric substance of polysaccharides, lipids, proteins and nucleic acids **(Younis *et al*., 2019)**. Biofilms and microorganisms have a difficult eradication because they are a form of protection against adverse environmental conditions and a form of resistance to antimicrobial agents in their growth and survival; therefore, in food production, cleaning and disinfection programs must be implemented for disposal **(Venegas *et al*., 2009; Novoslavskij *et al*., 2016; Younis *et al*., 2019).**

2. Fish

Fish is the one used for human consumption, encompassing all its portions and derivatives **(Silva *et al*., 2017)**. The source of fish intended for human consumption comes from capture fisheries and aquaculture; being the total fish production, reached in 2016, of 171 million tons, from which 88% were used for direct human consumption **(FAO, 2018)**.

Fish is considered a healthy food of high nutritional value for humans, being a source of high digestibility and biological value proteins, as well as the content of lipids (polyunsaturated fatty acids), vitamins and minerals **(Huss, 1999; Soares & Gonçalves, 2012; Samanta & Choudhary, 2019).**

Due to its composition and nutritional value, pH close to neutrality, and high water activity in tissues, fish is a food of animal origin that is very susceptible to deterioration due to activity of microorganisms, autolytic activity, oxidation and lipid hydrolysis, affecting its quality and safety **(Huss, 1999; Soares & Gonçalves, 2012).**

The fish is susceptible to chemical (natural toxins, heavy metals, industrial waste, cleaning chemicals, pesticides and antibiotics), and biological contamination at any stage of the food chain influencing factors such as microbiological water quality, environmental conditions, practices of production, handling, processing, conservation and distribution **(Romero & Negrete, 2011; Novoslavskij *et al*., 2016; WHO, 2016).**

Microorganisms in fish including pathogens can generally be divided into two groups: the natives of aquatic habitats having the temperatura of a selective effect (*C. botulinum, Vibrio* sp., *Aeromonas* sp.*, Plesiomonas* sp.*, Listeria monocytogenes*) and those associated with water pollution (*Salmonella* spp.*, Shigella* sp.*, E. coli., Staphylococcus aureus, Listeria monocytogenes*) **(Huss, 1997; Novoslavskij *et al*., 2016).** Species of the genus *Listeria* and *Yersinia* are cataloged in the two types because they are aquatic environments and pollutants including wastewater and that from direct contamination of wild animals, livestock, and food **(Novoslavskij *et al*., 2016)**.

Fish and products are among the foods frequently involved in the transmission of diseases being considered vehicles of pathogens such as: *Campylobacter* spp.*, Vibrio* spp.*, Yersinia* spp., *Salmonella* spp.*, E. coli, Clostridium botulinum, Listeria monocytogenes*, among others, which in turn are considered the main causative agents of consumer diseases around the world. This can be determined with contribution to their origin where they may be subject to a risk of biological and chemical contamination, in addition to the way in which these products are consumed **(Esesarte, 2002; Romero & Negrete, 2011; Gauthier, 2015; Terentjeva *et al*., 2015; Novoslavskij *et al*., 2016)**.

In Cuba, in the province of Villa Clara and through the National Epidemiological Surveillance System for foodborne illnesses in the period from 2004 to 2008, 371 outbreaks of foodborne illnesses were reported, with a rate of 4.54 by 10 000 inhabitants; the main foods involved were sausages and meat products with 42.6% of the outbreaks, 13.7% cheese, and fish with 8.72% **(Lopez *et al*., 2013)**.

In the United States of America, the Centers for Disease Control and Prevention (CDC) in its annual report of surveillance of outbreaks of foodborne illnesses of 2017 in that country, indicated a total of 841 outbreaks with 14481 patients and 827 hospitalizations. 42% of the outbreaks were caused by bacteria, mainly *Salmonella* spp., *Clostridium perfringens, Campylobacter* spp.*,* and *Escherichia coli,* producing toxin-shiga, *Vibrio parahaemolyticus, S. Aureus*, among others; while in the category of food involved, 39% of the outbreaks correspond to fish, crustaceans and mollusks **(CDC, 2019)**. In the United States, epidemiological surveillance continually identifies five risk

factors for foodborne illnesses, mainly associated with hygiene conditions and practices in food preparation, specifically inappropriate temperature management, poor hygiene, improper cooking, contaminated food and equipment **(Li, 2015)**.

In Spain, outbreaks of foodborne diseases reported by the National Epidemiological Surveillance Network, in the 2008-2011 period, were 2,342 in which 79.0% of them bacteria (*Salmonella* spp*., Staphylococcus aureus, Campylobacter* spp*., Clostidrium perfringens*, among others) were the main causal group in 69.5% of the outbreaks. Some foods were indicated, among which were eggs, egg products, and mayonnaise with 24.6% of the total, followed by meat and meat products (8%), shellfish (7.4%), fish and products (6.5%); to be contributed to factors that are poor hygiene practices such as cross contamination, processing time or inadequate temperature, and contaminated food **(Espinosa *et al*., 2015).**

3.   Regulation of food in the prevention of foodborne diseases (yersiniosis).

In the European Union, and due to its high incidence, the yersiniosis is classified as a surveillance zoonosis depending on the epidemiological situation of each country, and especially in those foods of risk, this in accordance with the provisions of Directive 2003/99 / EC of the European Parliament and of the Council **(Directive, 2003; Jimenez, 2018).**

Likewise, the European Commission on Food Safety Issues established Regulation (EC) No. 2073/2005 of the commission, regarding microbiological criteria applicable to food products including fish (*Salmonella, Listeria monocytogenes, Escherichia coli, Enterobacter sakazakii*, Staphylococcal Enterotoxin and Histamine). Such microbiological criteria serve as guidance on the acceptability of food products and manufacturing, handling and distribution processes. They also intervene in the application of hygiene control measures including the principles of Hazard Analysis and Critical Control Points (HACCP). However, although this regulation does not mention other pathogens such as *Yersinia* spp*., Campylobacter* spp., or *Vibrio* spp*.*, it does not mean that they are not considered for what general criteria should be applied, in accordance with the provisions of article 14 of the regulation (EC) 178/2002 in the matter of Food Safety in order to guarantee the non-commercialization of contaminated food **(Ferrer, 2016)**.

On the other hand, Regulation (EC) 852/2004 on the hygiene of foodstuffs, establishes general rules on hygiene of foodstuffs, applied to all stages of the production, processing and distribution of food and exports. Meanwhile, regulations (EC) 853/2004, establishes specific rules of hygiene of food of animal origin, including fish.

Globally, international organizations such as WHO and FAO have issued recommendations, describing fundamental practices in aquaculture necessary to guarantee the production of safe and nutritious fish **(WHO, 2016).** Thus, through the

Codex Alimentarius, the code of practice for fish and fishery products (CAC / RCP 52-2003) has been issued, focused on all those entities involved in handling, production, storage, distribution of fish and fishery products.   This code describes prerequisite programs that include technological guidelines and essential hygiene conditions, including the implementation of a Hazard Analysis and Critical Control Points (HACCP) for the production of fish and fishery products safe for human consumption **(Codex, 2012)**.   On the other hand, due to the commercial globalization of food, different standards, guidelines or certification schemes such as the Global GAP, Safe Quality Food (SQF), British Retail Consortium (BRC), Global Aquaculture Alliance (GAA) / Aquaculture Certification Council (AAC), ISO 22000, among others, have been developed in order to guarantee food safety, quality and environmental sustainability in the production of aquaculture and fishery products **(RAA, 2019)**.

In Latin American countries like Mexico, for the monitoring and regulation of the hygiene and safety of food production, manuals of good practices focused on aquaculture have been issued **(SENASICA, 2019).**   In the sanitary regulation, the official standard "NOM-251-SSA1-2009" establishes the minimum requirements of good hygiene practices, and that includes the implementation of the HACCP system that must be observed in food process and its raw materials in order to avoid contamination throughout its process and to ensure food safety.

The specific sanitary regulation for fish and products (fresh, chilled, frozen and processed) is the "NOM-242-SSA1-2009" standard that establishes the sanitary requirements, including the analysis and microbiological limits (*Salmonella* spp*., Listeria monocytogenes, Escherichia coli*, among others) that the products must meet.   However, this last regulatory standard does not yet include microbiological criteria or limits to be met in this type of focused products for the *Yersinia* spp.

4.   Microbiological analysis

The microbiological analysis of food is an important measure in the monitoring of the sanitary quality of food, as well as the reduction of risks to public health due to the incidence of foodborne diseases **(Hernández *et al*., 2017).** Traditional microbiological analysis methods based on phenotypic identification characteristics, where microbial culture is feasible, are still considered the gold standard in the detection of pathogens in food, environmental and clinical samples **(Morris & Feeley, 1976; De Boer, 1995; Bou *et al*., 2011; Carrillo *et al*., 2011; Fàbrega & Vila, 2012; Kishore *et al*., 2012; Momtaz *et al*., 2013; Dekker & Frank, 2015; Gupta *et al*., 2015; Weagant & Feng, 2017; Fredriksson & Laukkanen, 2018).** For the Yersinia analysis, cold enrichment phases (4°C to 10°C / 1 to 3 weeks) and alkaline treatment (KOH) are usually incorporated to reduce accompanying microbiota and improve *Yersinia* isolation **(Morris & Feeley, 1976 ; De Boer, 1995; Carrillo *et al*., 2011; Fàbrega & Vila, 2012; Kishore *et al*., 2012; Momtaz *et al*., 2013; Dekker & Frank, 2015; Gupta *et al*., 2015;**

Weagant & Feng, 2017; Fredriksson & Laukkanen, 2018). Traditional methods use nutritious, enrichment, differential and selective culture media such as: phosphate-buffered saline with sorbitol and bile salts (PBSSB), tryptone soy broth (TSB), bile oxalate sorbose (BOS) broth, tryptic soy broth, Luria –Bertani bile salts irgasan broth supplemented with cefsulodin and novobiocin, blood agar, chocolate agar, SS (Salmonella-Shigella) agar, MacConkey agar, Cefsulodin-Irgasan-Novobiocin agar (CIN), chromogenic agars for *Y. enterocolitica* (YECA), among others, as well as the incorporation of different biochemical systems for the identification of isolates (Table 1) **(Morris & Feeley, 1976; De Boer, 1995; Carrillo *et al*., 2011; Fàbrega & Vila, 2012; Momtaz *et al*., 2013; Dekker & Frank, 2015; Gupta *et al*., 2015; Fredriksson & Laukkanen, 2018).**

Table 1. Biochemical tests for the identification of *Yersinia* pathogenic species **(Morris & Feeley, 1976; Ewing *et al*., 1978; Weagant & Feng, 2017).**

Test	*Y. enterocolitica*	*Y. pestis*	*Y. pseudotuberculosis*	*Y. ruckeri*	*V. cholerae*	*A. hydrophila*	*Proteus morganii*
Oxi	-	-	-	-	+	+	-
Urea	+	-	+	-	-	-(+)	+
Lac	-	-	-	-	+(L)	-(+)	-
Suc	+	-	-	-	+	+	-
Mot at 22°C	+	-	+	+	+	+	+
(LD)	-	-	-	-	+	-(+)	-
(OD)	+	-	-	+	+	-	+
(PD)	-	-	-	-	-	-	+

*Lysine descarboxylase (LD), Ornithine descarboxylase (OD), Phenylalanine deaminase (PD), Suc: Sucrose, Mot: Motility, Oxy:Oxydase, Lac: Lactose.

The methods developed for the microbiological analysis focused on the culture and isolation of *Yersinia* spp*.,* present some analogies in terms of analysis phases that include enrichment, planting in differential and selective agars, and biochemical conformation.

Around the world, governmental or non-governmental organizations have developed standardized methods of microbiological analysis for *Yersina* species in different types of samples. Some of those methods are test method number 9 for the isolation of *Yersinia enterocolitica* in The Microbiology Laboratory Guidebook (MLG) by the The United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS).

The U.S. Bacteriological Analytical Manual (BAM) of the Food and Drug Administration (US FDA) reported a detection method for *Yersinia enterocolitica* in foods, which presents a sample enrichment phase with Peptone Sorbitol Bile Broth (PSBB) at 10 °C / 10 days, followed by culture in MacConkey agar and CIN agar, and

subsequent confirmation of colonies through various biochemical tests (lysine, arginine, urea, among others) **(Weagant & Feng, 2017).**

The UK Standards for Microbiology Investigations, along with the National Health Service of England, has developed the method ID 21 for the analysis and identification of *Yersinia enterocolitica, Yersinia pseudotuberculosis* and *Yersinia pestis* in clinical samples, involving primary isolation in Cefsulodin, Irgasan, Novobiocin agar (CIN) and Blood Agar (BA) under aerobic conditions at 28-30°C for 24-48h. The identification is performed by characteristic colonial growth and subsequent biochemical, proteomic confirmation through the Matrix-assisted laser desorption / ionisation time of flight mass spectrometry (MALDI-TOF MS) or molecular pathway through the Polymerase Chain Reaction (PCR), and strain typing by Pulsed Field Gel Electrophoresis (PFGE), Multilocus Sequence Typing (MLST), Multiple-Locus Variable- Number Tandem-Repeat Analysis (MVLA) or Whole Genome Sequencing (WGS) **(PHE, 2015)**.
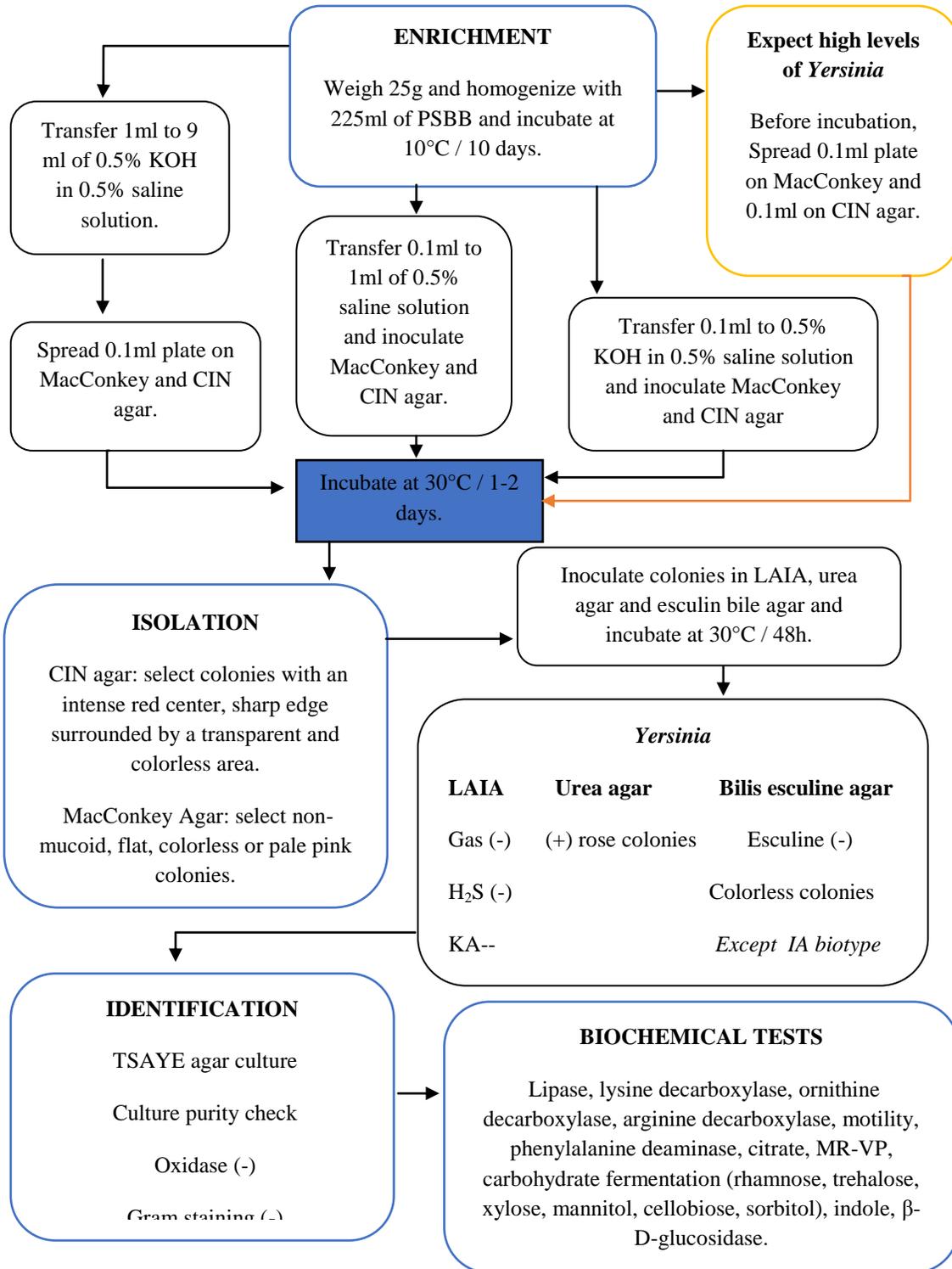
Finally, it is the method developed by the International Organization for Standardization (ISO) ISO 10273 for the microbiological analysis of the food chain with an *Y. enterocolitica* approach that was implemented in the Present study. The sample is placed in an enrichment with Peptone-Sorbitol-Bile broth (PSB), then an alkaline treatment is performed by adding 0.5% KOH in saline solution (0.5% NaCl) and striated in CIN agar, another recommended enrichment is in Irgasan-Ticarcillin-potassium Chlorate (ITC) broth and culture in Salmonella-Shigella-sodium-deoxycholate-calcium chloride (SSDC) agar by selecting characteristic colonies for confirmation through biochemical tests. Colonies in CIN agar are known as red ox eye, due to possessing a red center with a colorless translucent border **(Van Damme, 2013; Morka *et al*., 2018).** This method, in its 2017 version, presents the option to use chromogenic agars as well as alternative confirmation by PCR **(Sirghani *et al*., 2018).**

The traditional microbiological analysis of food has the disadvantage of being laborious and involving considerable time to obtain results, in addition to the fact that the sample transport process can affect the conservation of viable microorganisms in the sample, reflecting a low sensitivity **(Palomino & Gonzalez, 2014; Hernández *et al*., 2017)**. In addition, sometimes the absence of concordance between the observable or phenotypic characteristics of the isolation under study and those corresponding to the strain of the type species, make the traditional methods perform the most probable and not definitive identification **(Bou *et al*., 2011)**. Therefore, the development and implementation of alternative molecular methods has emerged through the Polymerase Chain Reaction (PCR) that allows greater efficiency, specificity, sensitivity, analysis of greater number of samples and rapidity in the detection time of microorganisms versus traditional methods **(Palomino & Gonzalez, 2014; Hernández *et al*., 2017)**. Molecular methods are based on the detection of 16S rDNA or genes that code for different virulence factors of *Yersinia* and other pathogens through the Polymerase Chain Reaction

(Carrillo *et al*., 2011; Momtaz *et al*., 2013; Palomino & Gonzalez, 2014; Bozcal *et al*., 2015; Gupta *et al*., 2015; Morka *et al*., 2018; Fredriksson & Laukkanen, 2018). Methods have also been developed for the typing and epidemiological studies of strains such as Pulsed Field Gel Electrophoresis (PFGE), Amplified Fragment Length Polymorphism (AFLP), Multilocus Sequence Typing (MLST), Random Amplification of Polymorphic DNA (RAPD) and Whole Genome Sequencing (WGS) **(Venegas *et al*., 2009; Fàbrega & Vila, 2012; Akhila *et al*., 2013; Dekker & Frank, 2015; Novoslavskij *et al*., 2016; Fredriksson & Laukkanen, 2018).** Proteomics-based methods have also been developed for the identification of Yersinia by Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) **(Bozcal *et al*., 2015; Gupta *et al*., 2015; Fredriksson & Laukkanen, 2018; Morka *et al*., 2018)**. Proteomic and molecular methods have begun to be incorporated as complementary in traditional methods but without replacing them, due to limitations such as those that are not yet widely incorporated in standardized methods, and require expensive equipment and reagents. The techniques are relatively complicated and they need expertise, use hazardous chemicals, the application of PCR, and other molecular methods in the detection and identification of pathogens in food emphasizes the presence of substances that can exert an inhibitory effect on the reaction making the results unreliable **(Bou *et al*., 2011; Palomino & Gonzalez, 2014).**

5. Control and prevention

Actions for the control and prevention of zoonoses through foods such as yersiniosis are considered, all along the food chain in agricultural, aquaculture and fisheries production, and in its different phases like the cultivation, breeding, fattening, slaughter, processing, transformation and conservation, vital for the application of good hygiene practices, as well as Hazard Analysis and Critical Control Points (HACCP) systems **(Elika, 2013; PAHO, 2019; Samanta & Choudhary, 2019).** While at the level of the general public or consumers, there is the avoidance of the use or consumption of untreated or potable water, raw or undercooked foods such as vegetables, meat and dairy products. In addition, good practices of food handling and preservation hygiene should be implemented (time-temperature), avoiding insufficient heating or cooking, cross contamination, poor cleaning and disinfection of hands, surfaces and utensils **(Gonzalez & Quiñones, 2005; WHO, 2007; CDC, 2016; Jimenez *et al*., 2018; PAHO, 2019)**.

ENRICHMENT

Weigh 25g and homogenize with 225ml of PSBB and incubate at 10°C / 10 days.

Transfer 1ml to 9 ml of 0.5% KOH in 0.5% saline solution.

Expect high levels of *Yersinia*

Before incubation, Spread 0.1ml plate on MacConkey and 0.1ml on CIN agar.

Transfer 0.1ml to 1ml of 0.5% saline solution and inoculate MacConkey and CIN agar.

Transfer 0.1ml to 0.5% KOH in 0.5% saline solution and inoculate MacConkey and CIN agar

Spread 0.1ml plate on MacConkey and CIN agar.

Incubate at 30°C / 1-2 days.

Inoculate colonies in LAIA, urea agar and esculin bile agar and incubate at 30°C / 48h.

ISOLATION

CIN agar: select colonies with an intense red center, sharp edge surrounded by a transparent and colorless area.

MacConkey Agar: select non-mucoid, flat, colorless or pale pink colonies.

Yersinia

LAIA	Urea agar	Bilis esculine agar
Gas (-)	(+) rose colonies	Esculine (-)
H$_2$S (-)		Colorless colonies
KA--		*Except IA biotype*

IDENTIFICATION

TSAYE agar culture

Culture purity check

Oxidase (-)

Gram staining (-)

BIOCHEMICAL TESTS

Lipase, lysine decarboxylase, ornithine decarboxylase, arginine decarboxylase, motility, phenylalanine deaminase, citrate, MR-VP, carbohydrate fermentation (rhamnose, trehalose, xylose, mannitol, cellobiose, sorbitol), indole, β-D-glucosidase.

*Peptone Sorbitol Bile Broth (PSBB), Celfsulodin-Irgasan-Novobiocin (CIN) agar, Trypticase Soy Agar with Yeast Extract (TSAYE), Lysine Arginine Iron Agar (LAIA). KA: alkaline reaction, acid. H$_2$S: sulfidric acid. (+) positive reaction, (-) negative reaction.

Fig. 1. Method for the isolation of *Yersinia enterocolitica* in food, water and environmental samples (**Weagant & Feng, 2017**).

6. Antimicrobial resistance

Antimicrobial resistance is considered a global threat and a challenge to human and animal health due to the treatment of infections acquired in both the community and hospitals; in addition, its effects have been extended to food safety, food security and economic well-being **(Tafur *et al*., 2008; Duarte & Granados, 2012; Codex, 2019).**

Food plays an important role in the development and spread of antimicrobial resistance. The presence of antimicrobial resistant microorganisms, many of them pathogenic in the food chain, is a potential route of exposure and health risk **(Codex, 2019).**

Resistance is understood as the mechanism by which the microorganism, mainly bacteria, can decrease the action of antimicrobial agents; this resistance can be intrinsical or adaptive and transmitted horizontally (same microbial genus) or vertical (different microbial genus) by means of conjugation, transformation, transposition, and transduction phenomena **(Crespo, 2005; Cabrera *et al*., 2007; Duarte & Granados, 2012).** Bacteria have developed several mechanisms to resist the action of antibiotics. These mechanisms are as follows: 1. Enzymatic modification of the antibiotic, 2. Expulsion pumps, 3. Changes in the permeability of the outer membrane, 4. Alterations of the site of action, and 5. Production of biofilms **(Cabrera *et al*., 2007; Tafur *et al*., 2008; Duarte & Granados, 2012; Andersen *et al*., 2015**).

The excessive and inappropriate use of antimicrobials in human medicine and agricultural, livestock and aquaculture activities, where in the latter those compounds are used for therapeutic, prophylaxis and promotion of animal growth, has led to changes in bacterial ecology, leading the phenomenon of resistance to have serious consequences for public health **(Crespo, 2005; Cabrera *et al*., 2007; Peña *et al*., 2011; Duarte & Granados, 2012; Codex, 2019**).

The treatment of *Yersinia* infection does not require treatment in immunocompetent hosts since infections are self-limited and only involves hydration and nutritional support. The antimicrobials of choice are aminoglycosides, trimethoprim-sulfamethoxazole or doxycycline, ciprofloxacin, ceftriaxone, cefotaxime; others may be tetracycline (not in children), and quinolones **(Dekker & Frank, 2015; Aziz & Waheed, 2019; Younis *et al*., 2019).** Since antibiotics are used only in selected patients (elderly, immunocompromised people or with diabetes), systemic, extraintestinal and invasive infections with a higher risk of developing bacteraemia or septicemia require special attention and antibiotic therapy, with the mortality rate in these cases up to 50% **(Aziz & Waheed, 2019; Younis *et al*., 2019).**

Antimicrobial resistance by various pathogenic enterobacteria such as: *E. coli, K. pneumoniae, Yersinia enterocolitica, Enterobacter* spp*., Salmonella* spp. has been

reported worldwide (**White** *et al*.**, 2002; Crespo, 2005; Cabrera** *et al*.**, 2007; Peña** *et al*.**, 2011; Andersen** *et al*.**, 2015; Fredriksson & Laukkanen, 2018**).

Yersinia strains are commonly resistant to β-lactam antibiotics due to genes on chromosomes that code for β-lactamases (**Fredriksson & Laukkanen, 2018**). *Y. enterocolitica* has shown β-lactamases, which confer resistance to ampicillin, cephalothin and carbenicillin; in addition, significant resistance to fluoroquinolones has been reported, due to the mutation of the *gyr* A gene and outflow mechanisms (**Dekker & Frank, 2015**).

The isolation of *Yersinia enterocolitica* strains from food with antimicrobial resistance has been reported around the world through various studies. Just to name a few cases, **Lee** *et al.* (**2004**), when analyzing ready-to-eat plant-based foods from supermarkets in Chunchon, South Korea obtained the isolation of strains with antimicrobial resistance (Ampicillin, Cephalothin and Carbenicillin).

Ye *et al*. (**2015**) obtained 70 strains of *Y. enterocolitica* from frozen meat samples (duck, pork, chicken, beef and sheep) and frozen pasta marketed in different provinces of China when performing the antimicrobial susceptibility test. The previous authors found that 69 % of the strains were ampicillin resistant, 20% resistant to amoxicillin / clavulanic acid 2:1, 67% resistant to cephalothin, 9% resistant to chloramphenicol, 13% resistant to nalidixic acid, and 52% resistant to trimethoprim / sulfamethoxazole.

Younis *et al*. (**2019**) collected 210 samples of processed poultry and beef from different retail outlets and markets located in the city of Mansoura, Egypt, obtained the isolation of 30 strains of *Yersinia enterocolitica* from the total samples. When performing the antimicrobial sensitivity analysis, the researcher found that 50% and 83% of the strains were resistant to streptomycin, cephalothin and ampicillin, respectively, by the mechanism of beta-lactamase synthesis.

For foods of aquatic origin **Kishore** *et al*. (**2012**), by microbiological analysis of fish, crustaceans and molluscs from aquaculture farms, fishmongers and landing centers on the southwest coast of India, obtained the isolation of *Y. intermedia*, followed by *Y. aldovae, Y. rohdei, Y. bercovieri, Y. enterocolitica, Y. kristensenii, Y. pseudotuberculosis and Y. frederiksenii*. When performing the antimicrobial susceptibility test to the *Y. enterocolitica* strains, the researcher reported that they were resistant to ampicillin, cephalothin and sulphamethizole. Meanwhile, in fish products marketed in the city of Coimbatore in India, **Akhila** *et al.* (**2013**) reported the isolation of *Y. enterocolitica* strains resistant to amoxicillin, ampicillin, amoxyclav, erythromycin, methicillin, nalidixic acid, polymyxin-B, rifampicin, and tetracycline mainly considering it a priority issue of public health and significant impact of geographic location, local selective pressure in the determination of antibiotic resistance of food pathogen isolates.

In all the case studies the researchers conclude that those foods form a radical source of danger to the health of consumers and recommend, for the generation of safe foods, the implementation of hygiene practices, microbiological control along the food chain, in addition to the proper use of antibiotics in animal production. Studies focused on the surveillance of antimicrobial sensitivity in foodborne pathogens are a vital tool to implement and improve the treatment against infections derived from the consumption of contaminated food.

Among the actions that have been recommended globally, in order to minimize antimicrobial resistance, are the use and rational practices of antimicrobial agents in human medicine, agricultural activities and veterinary medicine. The latter was recommended with emphasis on the production of food of animal origin, as well as the establishment and optimization of surveillance programs in the detection of sanitary quality indicator strains, hygiene parameters, resistant strains, and improvement of methods to determine antimicrobial susceptibility **(Peña *et al*., 2011; Pérez & Robles, 2013; Codex, 2019).** The implementation of good hygiene practices in the food production chain is considered essential for food safety and the fight against antimicrobial resistance **(Codex, 2019).**

CONCLUSION

Foodborne diseases are a challenge and a serious health problem worldwide, derived from their incidence, mortality and negative effects on public health.

Yersinia enterocolitica is a human food pathogen that causes a zoonosis called yersiniosis, which has a high incidence worldwide derived from the consumption of food contaminated mainly in the raw state or subjected to inadequate conditions of hygiene, cooking and conservation.

Fish is considered a highly nutritious and widely consumed food in different culinary forms including its raw state. However, this food is also very susceptible to contamination by various pathogenic microorganisms such as *Y. Enterocolitica*, being commonly related as one of the main causal foods of numerous outbreaks of foodborne illness in various parts of the world.

It has been determined that the control and prevention measures of foodborne diseases, and in this case of fish throughout all phases of the food chain (from the farm to the consumer's table), is the implementation of good hygiene practices, safety management systems such as HACCP, as well as regulation and surveillance through microbiological analysis of food.

The phenomenon of antimicrobial resistance is a health issue of great concern worldwide. The food chain plays an important role in the spread of this phenomenon by being a source of spread and exposure to pathogens. Various microorganisms globally like *Y. enterocolitica* have been isolated from different foods, such as fish, and show resistance to various antimicrobials including some that are used in the treatment of infections. It has been established that actions such as the regulation and proper use of antimicrobials in human and veterinary medicine, as well as the implementation of good hygiene practices in food production, monitoring of the sanitary quality of food and antimicrobial resistance by strains, carry a relevant function to control antimicrobial resistance and treatment of potential infections.

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