

## *Helicobacter pylori*, food, fish and tilapia

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### Abstract

Food safety is considered an essential characteristic of food quality and is one that establishes that food, when ingested, will not cause harm to the consumer's health. Foodborne diseases are considered a health problem worldwide due to their incidence and mortality; these diseases have different causal agents of a physical, chemical and biological nature, being the latter mainly related to disease outbreaks where they are included (viruses, bacteria, parasites, and fungi), generally affecting low-income population groups, children, the elderly, pregnant women, and immunosuppressed. This review focused on giving a general description of food-borne diseases, mainly those developed in fish, highly nutritious food that is highly susceptible to deterioration and contamination by various chemical and biological hazards. Among the fish with the highest production for marketing and consumption is tilapia, which can be a vehicle for transmission of various pollutants of biological origin, including *Helicobacter pylori*, which has been considered an emerging food pathogen with a considerable impact on human health, whose food contamination and infection may be due to different factors such as inadequate hygiene practices at different stages of the food chain, putting the safety of these foods, and/or the health of consumers at risk.

## 1. Introduction

Food safety is that characteristic consisting of not causing harm or disease to people who consume it. It is considered, along with the nutritional, organoleptic and commercial characteristics, one of the components of the total quality of food (Fuente-Salcido and Barboza-Corona, 2010). Safety is considered a complex aspect with high impact on social edges such as consumers, governments, the food industry, and academia (Jorquera *et al.*, 2015).

Contamination by physical, chemical and/or biological agents that put food safety at risk can occur at any stage of the food chain, going from primary production to final preparation prior to consumption (from farm to fork), thus generating diseases (Fuente-Salcido and Barboza-Corona, 2010; WHO, 2020).

Foodborne illness is considered a public health challenge worldwide due to its incidence and mortality, the socio-economic burden due to high levels of detriment in productivity, costs in health services,

implementation, surveillance and control of the food safety (Olea *et al.*, 2012; Alerte *et al.*, 2012; Torrens *et al.*, 2015). These diseases are the product of ingesting food and/or water contaminated with chemical or biological agents in amounts that affect the consumer's health (Torrens *et al.*, 2015; Soto Varela *et al.*, 2016; WHO, 2020).

It is estimated that these diseases affect 1 in 10 people each year worldwide, and generate 420,000 deaths, especially children under 5 years old (WHO, 2020). The general symptoms of these diseases are gastrointestinal, like nausea, vomiting, diarrhea, abdominal pain, and fever; being able to present complications such as sepsis, meningitis, abortions, Reiter's syndrome, Guillan Barré syndrome, cancer, or even death (WHO, 2020). These diseases mainly affect population groups such as children, pregnant women, the elderly, and people with poor immune systems, generally subjected to low socioeconomic levels and high levels of unhealthiness (Olea *et al.*, 2012; WHO, 2020; Soto Varela *et al.*, 2016; Torrens *et al.*, 2015).

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The increase in cases of food-borne illnesses is associated with factors such as market globalization, the introduction of new products and manufacturing processes, changes in society's eating habits, consumption of packaged foods, meals outside the home, sale of meals prepared and fast foods. It is also believed that socio-demographic factors, such as the aging of the population, may contribute to the appearance (Olea et al., 2012; Jorquera et al., 2015).

Contaminants in foods are varied over 250 causative agents of foodborne illness have been described. Contaminants can have a chemical nature (antimicrobials, growth promoters, food additives, natural toxins, disinfectants, heavy metals, pesticides among others), physical nature (fragments of glass, metal, wood, or others) or biological nature (bacteria, parasites, viruses and prions), the latter being mainly bacteria such as *Salmonella* spp., *Clostridium* spp., *Vibrio* spp., *Aeromonas hydrophila*, *Campylobacter jejuni*, *Helicobacter pylori*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*; those are among the confirmed cases and disease outbreaks (Hernández et al., 2010; Olea et al., 2012; Torrens et al., 2015; Soto Varela et al., 2016).

This review focuses on giving a general description of foodborne illness, particularly fish, highly nutritious food that is highly susceptible to deterioration and contamination by various chemical and biological hazards. Among the fish with the highest production for marketing and consumption is tilapia, which can be a vehicle for transmission of various pollutants of biological origin, including *H. pylori*, which has been considered an emerging food pathogen with a considerable impact on human health, whose food contamination and infection may be due to different factors such as inadequate hygiene practices at different stages of the food chain, putting the safety of these foods and the health of consumers at risk.

## 2. Fish

Fish is all that fish extracted from oceanic or continental waters destined for human or animal consumption (Soares and Gonçalves, 2012). The sources of fish destined for human use and consumption come from fishing and aquaculture activities, reaching in 2016 a combined world production of 170.9 million tons, and a per capita consumption of 20.3 kg (FAO, 2018). Fish is considered a highly nutritious food (a source of proteins of biological value and digestibility, lipids mainly unsaturated fatty acids, vitamins and minerals) widely marketed and consumed (Soares and Gonçalves, 2012; FAO, 2018).

Due to its chemical composition and slightly acidic pH of its meat, fish is a food highly susceptible to deterioration derived from the endogenous autolytic activity, microbial and chemical (oxidative) activity, and at the same time, prone to contamination by various physical, chemical and biological agents, due to inadequate hygiene practices throughout the different phases of the food chain, compromising quality and safety and causing a health risk to consumers (Pascual Anderson and Calderon y Pascual, 2000; Soares and Gonçalves, 2012; Ampuero et al., 2018).

Among the dangers and causative agents of diseases due to fish consumption, are those mainly of chemical and biological origin. Table 1 shows the different dangers that compromise safety and put human health through fish at risk.

In the case of microbiological activity and development related to deterioration and contamination of fish associated with human diseases, we will point out that, in captured and alive fish, the microbiota is found in the skin, gills and intestines; the number and type will depend on the time of year, feeding characteristics, geographical area, fish species, capture system and environmental conditions (Huss, 1999; Ramírez et al., 2011). The skin, gills and intestines are organs in direct contact with the muscle, and constitute the beginning of the bacterial invasion, so there will be a proportional correlation between the number of microorganisms in these organs and the muscle in the edible portion (Fuentes et al., 2011). In the case of biological agents, such as the bacteria responsible for the deterioration and causing diseases due to fish consumption, two types are grouped together: a) Autochthonous: those associated with the aquatic environment where they live, such as *Pseudomonas* spp., *Moraxella* spp., *Shewanella* spp., *Vibrio* spp., *Listeria* spp., *Lactobacillus* spp., *Clostridium botulinum*, *Plesiomonas shigelloides*, *Aeromonas* spp., among others where water temperature has a selective effect on its presence, and b) Non-autochthonous bacteria associated with fecal contamination of the aquatic environment and a poor hygiene and sanitation conditions in the different stages of production, processing, distribution and storage, such as *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, among others (Huss, 1997; dos Santos, 2010; Romero-Jarero and Negrete-Redondo, 2011; Ramírez et al., 2011).

### 2.1 Tilapia

Tilapia is a freshwater fish originating in Africa; they belong to the *Cichlidae* family and are classified

Table 1. Different pollutants and health hazards transmitted by fish

Type / Origin	Classification	Pollutant / Hazard	References		
Biological	Parasites	Members of families: <i>Opisthorchiidae Heterophyidae</i> <i>Paragonimidae</i> <i>Anisakidae</i> <i>Gnathostomidae Diphyllbothridae</i> <i>Cryptosporidiidae</i> <i>Hexamitidae</i>	Huss, (1997); Quijada <i>et al.</i> (2005); dos Santos (2010); Soares and Gonçalves (2012); Cortés-Sánchez (2020); Londoño-Bailón <i>et al.</i> (2020)		
		Bacteria	<i>Vibrio parahaemolyticus, V. cholerae, V. vulnificus, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Salmonella spp., Shigella spp., Streptococcus spp., Escherichia coli, Staphylococcus aureus, Bacillus cereus, Campylobacter jejuni, Yersinia enterocolitica, Aeromonas hydrophila, Edwardsiella tarda</i> among others.	Huss, (1997); dos Santos (2010); Romero-Jarero and Negrete-Redondo, (2011); Cortés <i>et al.</i> (2019); Puig <i>et al.</i> (2019); Sanabria Sánchez and Chiquillo Pompeyo (2019); Cortés-Sánchez (2020)	
			Virus	Virus Hepatitis A, Hepatitis E, Adenovirus, Norovirus, Astrovirus, Enterovirus.	Huss, (1997); Soares and Gonçalves (2012); Cortés-Sánchez (2020)
			Fungi	Mycotoxins of genus <i>Claviceps</i> spp., <i>Fusarium</i> spp., <i>Aspergillus</i> spp., and <i>Penicillium</i> spp.	Anater <i>et al.</i> (2016); Cortés-Sánchez <i>et al.</i> (2016); Cortés-Sánchez (2020)
Chemical	Biotoxins	Biogenic amines (Histamine, putrescine and cadaverine). Tetrodotxin, Ciguatera (ciguatoxin, scaritoxin, maitotoxin, palytoxin and okadaicacid) gempilotoxin.	Huss, (1997); Izquierdo <i>et al.</i> (2004); dos Santos (2010); Cortés-Sánchez (2020)		
	Heavy metals	Lead, cadmium, copper, mercury, arsenic, selenium, aluminum and strontium	Huss, (1997); Gonzalez <i>et al.</i> (2009); dos Santos, (2010); Azmat <i>et al.</i> (2012); Mahalakshmi <i>et al.</i> (2012); Estupiñán and		
	Organical Compounds	Antibiotics, hormones, Polychlorinated biphenyls, polybrominated diphenyls, polycyclic aromatic	Huss, (1997); dos Santos (2010); Yu <i>et al.</i> (2011); Cortés-Sánchez (2020)		
	Pellets or micro-plastics	Monomers and/or polymers (bisphenol-A , phthalates, polybrominated diphenyl ethers, polyethylene, polypropylene, polystyrene, Polyvinyl Chloride (PVC), Ethylene-vinyl acetate (EVA) and others	Miranda and de Carvalho-Souza (2016); Forrest and Hindell (2018); Smith <i>et al.</i> (2018)		

into three genera that differ from each other according to the patterns of parental care: *Tilapia*, *Oreochromis*, and *Sarotherodon* (Jácome *et al.*, 2019). The genus *Oreochromis* (*O. niloticus*, *O. aureus*, *O. mossambicus*), and interspecific hybrids (red tilapia), have been suitable organisms for aquaculture activities and commercialization worldwide because they tolerate high densities, have a fast growing, are resistant to diseases, are adaptable in captivity, accept balanced diets, in addition to their meat being of acceptable quality and affordable price (Baltazar, 2007; Jácome *et al.*, 2019).

Only in 2016, tilapia (*O. niloticus*) represented the second place in the total production of fish worldwide, with 8% being Asian countries like China among the main producers (FAO, 2018). Most of the tilapia, whether captured or cultivated, is consumed fresh,

frozen, fillets and/or smoked. In addition, in several tropical and subtropical countries, tilapia is being appreciated as a source of quality animal protein for human consumption, even for its lean and odorless state. It has been proposed as an alternative for the development of ready-to-serve foods such as fish curry, sandwiches, chops, hamburgers, and sticks (Dhanapal *et al.*, 2010). However, tilapia, as a fish destined for human consumption, has been identified as vulnerable to contamination by various biological hazards in specific bacteria such as *Aeromonas* spp., *Pseudomonas* spp., *Vibrio* spp., *L. monocytogenes*, *Staphylococcus aureus*, *Helicobacter pylori*, *Escherichia coli*, and various genera of the coliform group throughout the food chain that might put consumers' safety and health at risk (Gutiérrez, 2001; Gutiérrez *et al.*, 2010; Fuentes *et al.*, 2011; Ramirez *et al.*, 2011; Fuentes *et al.*, 2011; Goja *et al.*,

2013; Junior et al., 2014; Abdel-Moein et al., 2015; Eltholth et al., 2018).

### 3. *Helicobacter pylori*

The *Helicobacter* genus is extensive, at least thirty species isolated from the gastrointestinal tract of many animals have been identified. Some of the species include *H. acinonychis*, *H. acinonyx*, *H. bile*, *H. canis*, *H. cetorum*, *H. cholecystus*, *H. felis*, *H. hepaticus*, *H. muridarum*, *H. nemestrinae*, *H. pametensis*, *H. pullorum*, *H. trogonum*, *H. pylori*, *H. cinaedi*, *H. fennelliae*, and *H. mustalae* (Buller, 2004; Romero Cabello, 2007). *Helicobacter pylori*, from the epidemiological point of view and importance in human health, has a preponderant place, which among its characteristics are: it is a Gram negative bacterium with a spiral or helical shape, measuring 1.5 to 5 µm long and 0,3 to 1.0 µm in diameter, non-sporulated, microaerophilic (2-5% oxygen, CO<sub>2</sub> 10%, and N<sub>2</sub> 85%), mobile with two to six flagella; it has a genome of 1.6 Mb, and an average composition of G + C of 39%, it is catalase and positive oxidase, it also produces urease that allows it to tolerate gastric pH and survive, it is considered a demanding bacterium, since it requires supplemented means for its growth, its growth pH is 4.5-9, it is sensitive to environments with low water activity (aw) where growth is inhibited at values <0.98 and is inhibited at NaCl concentrations ≥2.5 g/L, the growth temperature is 34-40°C, incubation usually lasts between 3 and 7 days; their colonial morphology is described as small, gray and slightly hemolytic colonies (van Duynhoven and Jonge, 2001; Majalca-Martínez, et al., 2001; Castillo-Rojas et al., 2004; Romero Cabello, 2007; Palomino and Tomé, 2012; Ghorbani et al., 2016; Cervantes-Garcia, 2016; Cervantes, 2016).

The *H. pylori* infection is considered of importance in aspects of global public health (Fernández-Delgado et al., 2008). *H. pylori* infection is estimated to affect more than 50% of the global population depending on their sanitary conditions (Cervantes-Garcia, 2016; Otero, 2017), being the highest prevalence in developing areas such as Africa (79.1%), Latin America (63.4%) and Asia (54.7%), in contrast to the lowest prevalence in North America (37.1%) and Oceania (24.1%). Being considered a public health problem (Cervantes, 2016; Otero, 2017), in Latin America, in countries such as Mexico, the prevalence of *H. pylori* infection is 66% (Paniagua-Contreras et al., 2007).

The infection begins with chronic gastritis that is defined as an infectious disease, which can lead to complications such as peptic ulcer, lymphoma of the lymphoid tissue associated with gastric mucosa (MALT), and gastric cancer; these last two developed in a minority

of infected people (1-5%) (Ramírez and Sánchez, 2008; Atapoor et al., 2014; Cervantes-Garcia, 2016; Cervantes, 2016; Otero, 2017). This microorganism is considered by the World Health Organization (WHO) as a type I carcinogen, and it is associated to 90% of gastric cancer cases; however, it must be considered that this type of cancer is a multifactorial entity where, in addition to *H. pylori*, requires other factors such as environmental factors (food) or host genetics, among others. Considering that most of those infected cases will not develop cancer, *H. pylori* is treated as a necessary cause, but not sufficient (Ramírez and Sanchez, 2008; Atapoor et al., 2014; Otero, 2017).

One of the main reservoirs of this bacterium is the human stomach, considering that the routes of infection can be oral-oral, fecal-oral, gastro-oral, sexual, and through contaminated food and water (Riveros et al., 2009; Palomino Camargo and Tomé, 2012; Abdel-Moein et al., 2015; Cervantes, 2016; Otero, 2017; Zamani et al., 2017; Bayona and Gutierrez, 2017; Shahi and Yan, 2018). Infection with this bacterium is usually acquired during childhood, associated with socioeconomic status, poor hygienic conditions, and a high degree of overcrowding (Fernández-Delgado et al., 2008; Cervantes, 2016).

The role of water and food in the transmission of *H. pylori* has not been established yet; however, its isolation has been reported in different foods such as dairy, meat, fish, vegetables and salads, especially raw or ready-to-eat, indicating that these foods are the source and are involved in the transmission, considering it a food pathogen (Hernández, 2010; Ghorbani et al., 2016; Atapoor et al., 2014; Hemmatinezhad et al., 2016; Zamani et al., 2017; Quaglia and Dambrosio, 2018). This bacterium can exist naturally in aquatic environments such as lakes, rivers, surface and underground drinking water, municipal and wastewater, and coastal marine environments, as a free microorganism or associated with planktonic organisms, being the water both reservoir and transmission source (Palomino Camargo and Tomé, 2012; Fernández-Delgado et al., 2008; Shahi and Yan, 2018). The infective dose in humans from water has been estimated to be 10<sup>4</sup>-10<sup>10</sup> CFU/L (Otero, 2017). *H. pylori* has the ability to form complex communities between microorganisms attached to surfaces or associated to interfaces, being called biofilms, and it also presents viable but non-cultivable forms (VBNC) where its cellular morphology is coccoid, as for adaptation and survival in conditions of stress due to nutritional situations, and unfavorable physicochemical environment (Palomino Camargo and Tomé, 2012; Giao et al., 2008; Fernández-Delgado et al., 2008; Otero, 2017; Ng et al., 2017; Zamani et al., 2017;

Bayona and Gutierrez, 2017; Quaglia and Dambrosio, 2018). These VBNC forms have been detected in the oral cavity and feces of the infected, meaning a source of transmission of the pathogen (Juneja and Sofos, 2010).

The pathogenicity and virulence mechanisms of this bacterium are varied. Among those, it can be found that they are structural lipopolysaccharides; the spirillated structure and flagella for interaction, mobility in gastric tissue, adhesins for colonization and tropism in gastric tissue, such as Lewis B antigens (BabA), inflammatory outer membrane protein (OipA), sialic acid binding adhesin (SabA), adhesion-associated lipoprotein (AlpA and AlpB), and HopZ, which prevent it from being carried away by peristalsis, ciliary activity, and epithelial turnover enzymes such as urease that transforms urea into ammonia, giving rise to an alkaline microenvironment and protecting it against gastric acidity, lipases, and proteases that contribute to the degradation of gastric mucus and loss of hydrophobicity of the mucosa, decreasing the ability of mucous cells to secrete mucus, catalase, and superoxide dismutase that protect the bacteria from oxidative processes of immune cells (Macro phages and neutrophils), in addition to phospholipase A2, phospholipase C, mucinase, alkaline and acid phosphatase (Dunn, 1997; Posse *et al.*, 2006; González López and Rodríguez González, 2011; Espinoza, 2012; Cervantes-Garcia, 2016). Different virulence factors of *H. pylori* have also been described, among which are vacuolizing cytotoxin encoded in the *vacA* gene (vacuolization associated gene) that varies in regions *s* and *m*, having *s1* or *s2* and *m1* or *m2* allelic types. This gene is present in all strains of *H. pylori* associated with damage to epithelial cells in the gastric tract and the *cagA* protein that may be involved in the activation of the vacuolizing toxin, and is encoded in the *cagA* gene (cytotoxin associated gene) present in 50%-70% of strains associated with peptic ulceration and gastric cancer (Castillo-Rojas *et al.*, 2004; Paniagua-Contreras *et al.*, 2007; Cervantes-Garcia, 2016; Ghorbani *et al.*, 2016; Morales *et al.*, 2018).

### 3.1 Prevention and control of food contamination and *H. pylori* infection

Among the main risk factors for *H. pylori* infection, it has been related to low educational level, low level of sanitation and hygiene, low health care, infected family members, low socioeconomic status, overcrowding, access to low-quality water sanitation, inadequate hygiene management and food contamination (van Duynhoven and Jonge, 2001; Zamani *et al.*, 2017; Bayona and Gutiérrez, 2017). *H. pylori* infection can be considered a zoonosis due to its detection in the oral and gastric cavity of animals in contact with humans such as

dogs, cats, cows and pigs, and its possible transmission is considered by the aforementioned animals (Palomino Camargo and Tomé, 2012; Bayona and Gutiérrez, 2017). Studies conducted on foods of aquatic origin, usually consumed minimally processed such as shellfish in Pina-Pérez *et al.* (2019), reported the detection of *H. pylori* in samples of shellfish (mussels, clams and cockles) from the Spanish west coast, confirming the possible relationship between the presence of *H. pylori* in contaminated seawater, and shellfish as vehicles for entry of the pathogen into the food chain and a risk to public health. Meanwhile, in fish such as tilapia, Abdel-Moein *et al.* (2015) detected the presence of *H. pylori* in both cultured specimens and in wild specimens captured from different aquatic environments, considering it a source of *H. pylori* transmission, and indicating that the fish may be a potential reservoir and transmitter of zoonosis. However, it also states that more studies of the pathogen occurrence in other fish are necessary.

In foods of meat origin with a high aw, pH, and optimal salt content, in addition to the presence of amino acids such as arginine, histidine, isoleucine, leucine, methionine, phenylalanine, alanine, valine, proline, serine, and tryptophan, the growth and presence of *H. pylori* is promoted, in addition to the fact that in temperatures below 30°C the bacterium can survive in fresh vegetables, fresh meats, and dairy products for several days, making it a source of transmission (van Duynhoven and Jonge, 2001; Palomino Camargo and Tomé, 2012; Ghorbani *et al.*, 2016; Zamani *et al.*, 2017; Ng *et al.*, 2017; Bayona and Gutierrez, 2017).

Another factor in the control and prevention of food contamination by *H. pylori* is the microbiological quality of the water. Drinking water and food production water must be subjected to adequate disinfection processes that eliminate the possibility of *H. pylori* transmission (Shahi and Yan, 2018). It has been reported that *H. pylori* in chlorine and ozone disinfection processes are more resistant than *E. coli* and *C. jejuni*. Therefore, inadequate disinfection conditions, such as the concentration of the disinfectant and the time of exposure, contribute to the persistence of the pathogen in the water and transmission in its cellular state, which can be in forms either spiral or coccoid (VBNC) (Johnson *et al.*, 1997; Baker *et al.*, 2002; Percival and Thomas, 2009; Orta de Velázquez *et al.*, 2016). In the United States, water service companies supply median residual chlorine of 1.1 mg/L, and an average exposure time of 45 minutes until the first use in the distribution system; this level of chlorination can be considered adequate to inactivate *H. pylori* (Johnson *et al.*, 1997).

In foodborne illnesses, fish has been related to

disease outbreaks, being the causal factors biological agents such as bacteria, where contamination due to lack or inadequate hygiene control at different stages of the food chain, such as production, transport, warehouse, handling, and processing are among the contributing factors (OMS, 2007; Ramírez *et al.*, 2011; Alerte *et al.*, 2012; Espinosa *et al.*, 2014; Soares and Gonçalves, 2012; OMS, 2016). Poor practices and hygiene conditions in food production, considering water and human reservoirs of *H. pylori*, favor contamination during food handling, preparation and the health risk (Palomino Camargo and Tomé, 2012; Atapoor *et al.*, 2014; Ghorbani *et al.*, 2016; Hemmatinezhad *et al.*, 2016; Shahi and Yan, 2018). Therefore, it has been stipulated that food safety standards that allow the safe production of products such as good agricultural practices, Good Aquaculture Practices for Fish Farming (GAP-FF), Good Manufacturing Practices (GMP), must be implemented in the production of food, as well as Sanitation Standard Operating Procedures (SSOP) and Hazard Analysis and Critical Control Points (HACCP) systems in the different phases of the food chain, and food hygiene education for final preparers or handlers (OMS, 2007; Ramirez *et al.*, 2011; Atapoor *et al.*, 2014; Jorquera *et al.*, 2015; Ghorbani *et al.*, 2016; Hemmatinezhad *et al.*, 2016; OMS, 2016).

### 3.2 Microbiological analysis of food

Conventional microbiological methods for pathogenic bacteria generally involve a laborious and time-consuming process in the proper use of enrichment culture media, and selective for subsequent biochemical, morphological and/or serological confirmation; in addition to that the target microorganism may present a viable but non-cultivable state (VBNC), which prevents or hinders the use of culture methods as a detection tool (Palomino-Camargo and González-Muñoz, 2014). Isolation and detection of *H. pylori* is difficult and infrequent, with recovery rates between 30-73% by conventional methods due to morphological changes of this bacterium due to not having conditions similar to the gastric epithelium that favor its colonization and growth, presenting a VBNC state, and the overgrowth or competition of other accompanying microorganisms (Fernández-Delgado *et al.*, 2008; Riveros *et al.*, 2009; Palomino Camargo and Tomé, 2012; Bernal and Cadavid Hincapié, 2013; Quaglia and Dambrosio, 2018).

Multiple investigations have been reported in the culture of this microorganism from various types of samples using different enrichment culture media, selective and differential such as agar and/or broth as Wilkins Chalgren agar, Brucella agar, Müeller Hinton agar, Brain Heart Infusion broth, Agar Skirrow,

Columbia Agar, and Soy broth trypticase applying in turn some of the different supplements (serum or horse blood, sheep blood, fetal bovine serum, yeast extract, lysed human erythrocytes, hemin, cyclodextrin, cholesterol, starch and/or nicotinamide adenine), antibiotics (trimethoprim, cycloheximide, vancomycin, nalidixic acid, colistin methanesulfonate, cefsulodin and/or amphotericin B) that favor the growth of the bacteria, and the use of different microaerophilic conditions, temperature between 35-37°C, with a time of incubation for 5 to 10 days, where the developed colonies are small grayish and shiny, are 1 mm in diameter, and are subsequently tested biochemical identification such as Gram staining, urease, cytochrome oxidase and catalase (Bode *et al.*, 1993; Majalca-Martínez *et al.*, 2001; Lu *et al.*, 2002; Buller, 2004; Fernández-Delgado *et al.*, 2008; Bermúdez *et al.*, 2009; Espinoza, 2012; Atapoor *et al.*, 2014; Mousavi and Dehkordi, 2014; Ghorbani *et al.*, 2016; Cervantes, 2016; Saeidi and Sheikhshahrokh, 2016; Quaglia and Dambrosio, 2018).

The difficulties presented by traditional cultivation techniques in recovering pathogenic microorganisms from the environment or food samples means that techniques such as Polymerase Chain Reaction (PCR), Fluorescence In Situ Hybridization (FISH), and immunofluorescence have been well established as an alternative for the detection of pathogens such as *H. pylori* (Fernández-Delgado *et al.*, 2008; Mousavi and Dehkordi, 2014; Atapoor *et al.*, 2014; Flores-Encarnación *et al.*, 2015; Ghorbani *et al.*, 2016; Cervantes, 2016; Quaglia and Dambrosio, 2018).

Detection through PCR in clinical, water and food samples is made by detecting genes that encode virulence factors such as cytotoxin (*vacA*), *cagA* gene, the *ureA* gene that encodes the A subunit of the urease enzyme, *glmM* gene coding for a phosphoglucosamine mutase, *oipA* gene involved in colonization of mucosa, and highly conserved sequences of the gene that codes for ribonucleic acid of the 16S subunit of the ribosome (16S rRNA), have shown good values of sensitivity and specificity in use (Lu *et al.*, 2002; Paniagua-Contreras *et al.*, 2007; Meng *et al.*, 2008; Bermúdez *et al.*, 2009; Mousavi and Dehkordi, 2014; Ghorbani *et al.*, 2016; Saeidi and Sheikhshahrokh, 2016; Hemmatinezhad *et al.*, 2016).

### 3.3 Antimicrobial resistance

Antimicrobial resistance is considered a major threat and challenge to human and animal health worldwide (Puig Peña *et al.*, 2019; FAO/WHO, 2020). Antimicrobial resistance is defined, from a clinical perspective, as the disappearance of response and a failure in the treatment of the disease to eliminate the

causative agent before the administration of antimicrobials, increasing morbidity, mortality and cost of medical care (García *et al.*, 2014; Puig Peña *et al.*, 2019). In the field of food, these intervene in the development and spread of antimicrobial resistance, having an impact on food safety and food security; the presence of antimicrobial resistant microorganisms in the food chain is a potential route of exposure and transfer to humans (Puig Peña *et al.*, 2011; FAO/WHO, 2020).

It has been established that the factors related to the generation of this problem are due to a combination of various factors, such as the inappropriate use of antimicrobials in humans and animals, counting the latter with three functions: therapeutics, prophylaxis and growth promotion aimed at the production of food (Puig Peña *et al.*, 2011).

Microorganisms, including bacteria, acquire antimicrobial resistance capacity through different mechanisms such as the modification of membrane permeability, the extraction by pumping mechanisms, enzymatic inhibition, modification of the attack target, or an alteration of the composition of the cell wall (Tafur *et al.*, 2008; Puig Peña *et al.*, 2011, García Castellanos *et al.*, 2014)

The microorganisms are capable of transferring and exchanging genetic material (plasmids, insertion sequences, integrons, and transposons), allowing genetic recombination, through transformation, transduction, or conjugation (Sánchez-B *et al.*, 2012). Therefore, developing different resistance mechanisms, and increasing the spread of this phenomenon (Puig Peña *et al.*, 2011). In the case of food from aquatic environments such as aquaculture, they are a means for microorganisms, including pathogens, to exchange resistance genetic material, constituting a risk for human and animal health (Puig Peña *et al.*, 2019).

Food-borne disease-causing bacteria have already been reported in various studies to show resistance to different antimicrobials, including *Escherichia coli*, *Listeria monocytogenes*, *V. cholerae*, *H. pylori*, *Staphylococcus* spp., *Campylobacter* spp., *Aeromonas* spp., among others (Puig Peña *et al.*, 2011; De Oliveira *et al.*, 2013; Hemmatinezhad, 2016; Cáceres *et al.*, 2017; Puig Peña *et al.*, 2019).

Treatments for *H. pylori* infection are: acid-suppressing drugs (proton pump inhibitors or H<sub>2</sub> receptor antagonists) such as bismuth salts, ranitidine, omeprazole, and antimicrobials such as amoxicillin, macrolides (clarithromycin), tetracycline, and nitroimidazole (metronidazole) (Posse *et al.*, 2004; Gerrits *et al.*, 2006; Romero Cabello, 2007; Hernández,

2008; Cervantes, 2016). Due to the serious consequences of an *H. pylori* infection, it must be detected and eliminated. However, there is a challenge in its elimination because the schemes for this purpose are less effective due to resistance of the microorganism to specifically clarithromycin antibiotics, so the World Health Organization (WHO) has included this pathogen among those that threaten humanity and that urgently merit measures for its control (Riveros *et al.*, 2009; Cervantes, 2016; Otero, 2017). Levofloxacin and ciprofloxacin are frequently used in second-line treatment; however, studies have been reported that strains resistant to these antibiotics have appeared (Gerrits *et al.*, 2006; Hernández *et al.*, 2008; Cervantes, 2016). Resistance to amoxicillin and nitroimidazole such as metronidazole is also common and high, reducing the response to treatment, which requires adjusting dose and duration (Gerrits *et al.*, 2006; Figueroa *et al.*, 2012; Cervantes, 2016).

The *H. pylori* resistance to different drugs used in the treatment of infection has been reported by different mechanisms, where they show that, for metronidazole, it is through mutations in the *rdxA* and *frxA* genes making them dysfunctional, and which encode NADPH-nitro reductase and NADPH-flavin oxide reductase respectively, avoiding drug activation (Hernández *et al.*, 2008). For amoxicillin, the resistance mechanism is mutations in the antimicrobial binding proteins causing changes in the amino acid sequence and conformations in reducing the affinity. Meanwhile, for tetracyclines, the resistance mechanism is due to mutations in the 16S *rrn* gene that codes for the 16S ribosomal RNA of the 30S subunit of the microbial ribosome, blocking the inhibition of protein synthesis by the drug. In addition, other mechanisms have been proposed, for example, the efflux or expulsion protein activity and reduced membrane permeability. Clarithromycin inhibits protein synthesis and the resistance mechanism is the result of point mutations in the 23S *rrn* gene that codes for the 23S rRNA of the 50S ribosomal subunit through substitutions of nitrogenous bases, leading to modifications in the structure of the ribosome, and affecting the binding of the antibiotic and, therefore, protein synthesis is not altered (Hernández *et al.*, 2008). And for levofloxacin, and ciprofloxacin that inhibits DNA replication by binding to DNA gyrase, the resistance mechanism is the mutation of the DNA gyrase subunit A (*gyr A*), replacing aspartate with glycine (D91G) and asparagine with lysine (N87K) (Hernández *et al.*, 2008).

In studies involved in the isolation of *H. pylori*, and in strains resistant to antimicrobials from foods such as those ready to eat ones (salads and soups), authors such

as Hemmatinezhad *et al.* (2016) have reported the analysis of 550 samples, where contamination by *H. pylori* occurred in 13.45% of those, and the isolated strains had high levels of resistance to antimicrobials such as amoxicillin (94.59%), ampicillin (93.24%), metronidazole (89.18%) and tetracycline (72.97%). Yahaghi *et al.* 2014 detected, in 380 samples of vegetables (radish, basil, parsley, spinach, lettuce, corn, broccoli, cucumber, among others) and 50 salads collected from supermarkets and grocery stores in Iran, the presence of *H. pylori* in 13.68% and 14%, respectively, of samples and the isolated strains presented high levels of antibiotic resistance against metronidazole (77.96%), amoxicillin (67.79%) and ampicillin (61.01%). However, they are sensitive to levofloxacin, rifampin, trimethoprim, cefsulodin, and spiramycin.

Mousavi and Dehkordi (2014) conducted studies on antimicrobial resistance on *H. pylori* strains isolated from raw milk samples and dairy products (cheese, cream, and ice cream) marketed in Iran, reporting high levels of contamination by this pathogen, (19.5%) and levels of resistance to ampicillin (84.4%), tetracycline (76.6%), erythromycin (70.5%) and metronidazole (70%). In all previous investigations, the authors conclude that in the detected strains there is high resistance to antimicrobials used in the treatment for infection of this pathogen, in addition to the fact that the consumption of contaminated food can constitute a potential health risk, in particular, due to the consumption of foods are mainly raw or produced under inadequate hygiene practices, which is why they suggest sanitary surveillance in food production (Mousavi and Dehkordi, 2014; Yahaghi *et al.*, 2014; Hemmatinezhad *et al.*, 2016). Among the measures implemented to face antimicrobial resistance is through the application of hygiene systems and procedures all along with the food production and supply chain (Puig Peña *et al.*, 2011; FAO/WHO, 2020). Control of antimicrobial resistance in food should be implemented in bacteria that indicate sanitary quality as hygiene parameters in addition to pathogens and zoonoses (Puig Peña *et al.*, 2011).

#### 4. Conclusion

Fish is considered a highly nutritious food, the main source of protein, produced through fishing and aquaculture activities for worldwide distribution and commercialization. Among the main fish producing species is tilapia (*O. niloticus*) due to its favorable aquaculture production conditions and market acceptance. However, fish as food is not only nutritious but also susceptible to deterioration and contamination by various biological hazards, mainly bacteria, such as *E.*

*coli*, *Salmonella* spp., *Vibrio* spp., *Listeria* spp., among others that are frequently related with the cause of gastrointestinal diseases in humans, mainly due to the fact that they are subjected to poor conditions and hygiene practices in their production and conservation.

*H. pylori* in recent years have gained relevance in the context of public health due to its high incidence, impact and resistance to antimicrobials. *H. pylori* affect a large part of the world's population and can be transmitted through the consumption of contaminated food and water such as fish. As the pathogen is detected in water, animals and humans make them a source of transmission, so it is necessary to monitor and control hygiene conditions in the production, conservation and handling of food throughout of the entire food chain in order to avoid various biological hazards including *H. pylori* to ensure the safety of food available for human consumption.

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