

**Review Article**

# Prevalence and distribution of pathogenic bacteria found in fish and fishery products: A review

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**ABSTRACT**

Fishes are among one of the major sources of food for many counties globally and a vital source of protein. Fishes are known to be carriers and vectors of pathogenic bacteria that are of major concern to consumers and public health. Contamination of pathogenic bacteria can arise from the aquatic ecosystem via pollution from domestic, industrial and agricultural discharges, contamination from soil, and also from the processing and marketing environments. Pathogenic bacteria that are associated with fishes and their related products include Gram negative bacteria like *Vibrio* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Pseudomonas* spp., *Listeria monocytogenes*, *C. botulinum* and *C. perfringens* dominating the micro-flora of fishes. Members of the Enterobacteriaceae family are among one of the most prevalent pathogenic bacteria isolated from fishes that pose serious health problems. Several parts of fishes including the skin/scales, flesh, intestines, and gills are among major areas that harbor these bacteria. Along with numerous factors, including freshness, spoilage and preservation, may influence the microbial loads and genera of bacteria in each part. Detection of pathogenic microorganisms or changes in natural micro-flora in the water and terrestrial (market) environment could be an important indicator of possible contamination. This provides insight on management practices that are utilized by fishermen and retailers to prevent contamination of their vulnerable products. Hence the aim of this paper was to review the prevalence and distribution of major pathogenic bacteria in fishes and their related products used for public consumption.

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**INTRODUCTION**

Seafood has traditionally been a popular part of human diet and vital as a main source of animal protein (Oramadike *et al.*, 2010; Osibona and Ezekiel, 2014; Akila and Kumaran, 2018). Fish is among one of the cheapest sources of protein after meat and is a major part of human protein supplement in many countries of the world (Latha and Mohan, 2013; Magbooljan and Kasturi, 2014; Reddy *et al.*, 2014; Shahriar *et al.*, 2019). An estimated 60% of the world protein is supplemented by fishes. As the world's population increases inevitably at a rate of almost 2% per year, the demand for seafood as a source of animal protein will increase (Begum *et al.*, 2010; Oramadike *et al.*, 2010; Razavilar *et al.*, 2012; Adedeji *et al.*, 2012; Velappan and Munuswamy, 2015; Huicab-Pech *et al.*, 2017).

The sea food resources in aquatic ecosystems are extremely vulnerable to pollution from domestic, industrial and agricultural discharges, contamination from soil, and airborne infections. Thus, the micro-flora of fresh fish are a

function of the micro-flora of the environment and act as indicators of the state of the environment and quality of the water from which these fishes are harvested (Olayemi *et al.*, 1990; Razavilar *et al.*, 2012; Reddy *et al.*, 2014; Velappan and Munuswamy, 2015; Jalal *et al.*, 2017; Shahriar *et al.*, 2019; Nur *et al.*, 2020).

Additionally, in areas where the water movement is very slow, bacteria can easily be transferred to fish from the water, sediments, and from its feeding behavior and food sources and populate the skin, gills and digestive tract, acting as one of the major vehicles for the transmission of pathogenic bacteria (Razavilar *et al.*, 2012; Latha and Mohan, 2013; Duarte *et al.*, 2014; Jalal *et al.*, 2017; Al-Sheraa, 2018; Barros-Velázquez *et al.*, 2019; Sasikala *et al.*, 2019; Nur *et al.*, 2020). The microbial contamination on environmental surfaces can be transferred to the fresh fish and its related products directly through surface contact or by vectors, pests or air movements. Fish can be infected

with bacteria during careless handling at landing sites, storage and through the cutting up process, thus leading to lower quality of fish and its related products (Strunjak-Perovic *et al.*, 2010; Latha and Mohan, 2013; Duarte *et al.*, 2014; Geetha *et al.*, 2014; Al-Sheraa, 2018; Sasikala *et al.*, 2019; Nur *et al.*, 2020).

It is a common belief among consumers that fishes caught fresh from the water source are free of pathogen and contamination (Binta *et al.*, 1982; Rusul and Mahyuddin, 1991). However, this may not be the case. Although fishes possess some amount of sterility, they have been found to be carriers of human pathogens such as those that are waterborne or as a result of secondary contamination such as sewage that can affect both the aquatic and terrestrial environment (Binta *et al.*, 1982; Abeyta, 1983; Razavilar *et al.*, 2012; Latha and Mohan, 2013; Pal *et al.*, 2016).

Several studies around the world have shown that that bacterial micro-flora of the skin and gut of fishes are highly susceptible to contamination from the aquatic environment, some of which can become pathogenic and aid in the spoilage if proper management practices are not in place (Novotny *et al.*, 2004; Latha and Mohan, 2013; Rokibul *et al.*, 2013; Ibemenuga and Okeke, 2014; Jalal *et al.*, 2017; Shahriar *et al.*, 2019). After death the mucus layer or slime coat which is a physical barrier that inhibits the entry of disease organisms from the environment into fishes is destroyed. The slime coat also possesses chemical properties since it is known to contain enzymes such as lysozymes and antibodies that can kill invading pathogens (Rusul and Mahyuddin, 1991; Razavilar *et al.*, 2012; Floyd, 2012; Latha and Mohan, 2013; Duarte *et al.*, 2014).

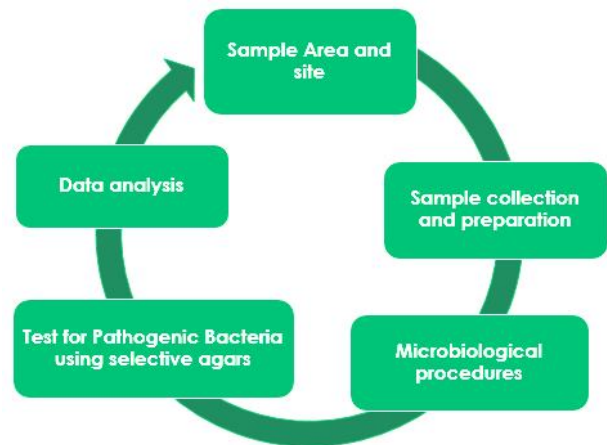
Many bacterial species encountered in different fishes are potentially pathogenic such as *Escherichia coli*, *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp., *C. botulinum*, *C. perfringens*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp. and *V. cholera* (Abeyta, 1983; Rusul *et al.*, 1991; Novotny *et al.*, 2004; Strunjak-Perovic, 2010; Adedeji *et al.*, 2012; Razavilar *et al.*, 2012; Sahu *et al.*, 2012; Pal *et al.*, 2016). Consumers along with health workers ought to be concerned about the hygienic quality of fishes that they consume (Binta *et al.*, 1982).

Hence this article seeks to review the prevalence and distribution of pathogenic bacteria that contaminate fishes, the areas of fishes that are affected and factors influencing pathogenic bacterial loads and contaminations and the related implications it may have on consumer

#### *Overview of methods used to detect pathogenic bacteria in fishes*

Over the years different methods have been used to detect pathogenic bacteria and in accordance with specific guidelines that are constantly updated based on current and new studies. It is vital that proper methods be in place to deal with identification, quantification and rapid assessment of pathogenic bacteria that many arise from different sources. The standardize test that are normally used to detect these bacteria usually involved testing a specific proportion (gram) of the samples (skin/flesh, intestines, gills), subjecting it to serial dilution and culturing against selective agar such as MacConkey agar, EMB agar, Mueller Hinton agar, Hektoen enteric (H.E) agar, Bismuth Sulfite (BS) agar, Xylose lysine desoxycholate (XLD) agar, Triple sugar iron agar and Lysine iron agar. Fig. 1 highlights a

typical method that is normally utilized to conduct a study of this nature.



**Fig. 1:** Typical Method Used to Conduct a Study of this Nature

Pathogenic Bacteria many be quantified using total plate count (TPC), total viable bacteria (TVB) and total coliform count (TCC). Bacterial identification is usually carried out using gram staining test, biochemical test such as carbohydrate tests (sucrose, glucose, fructose, lactose and mannitol), catalase test, coagulase test and indole test along with sensory analysis. Most recently, researchers have introduced the antibiotic resistance and susceptibility test to this category. This is largely because bacterial mutation and antibiotic resistance is among one of the growing problems in our current world. Hence, it is vital to understand how these pathogens interact and evolve in different conditions, as explained in many epidemiology studies (Oramadike *et al.*, 2010; Mgwede *et al.*, 2018; Shahriar *et al.*, 2019; Nur *et al.*, 2020).

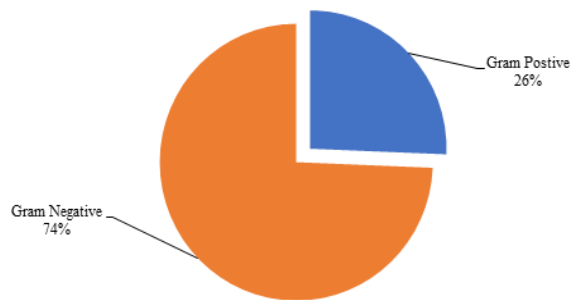
## RESULTS AND DISCUSSION

### *Pathogenies of Indigenous and Non-Indigenous Bacteria in Fishes*

Fresh fish is considered to be sterile, however several studies have shown that fishes and bacteria have a long history of association (Binta *et al.*, 1982; Rusul and Mahyuddin, 1991). Microorganisms have been found to colonize the skin, gills, and the gastrointestinal tract of fresh fish, with some being possibly pathogenic (Binta *et al.*, 1982; Razavilar *et al.*, 2012; Ibemenuga and Okeke, 2014, Giddings *et al.*, 2015; Akila and Kumaran, 2018; Nur *et al.*, 2020). In general, the natural fish micro-flora tends to reflect the microbial communities of the surrounding waters (Razavilar *et al.*, 2012; Latha and Mohan, 2013; Duarte *et al.*, 2014). Hence, human pathogenic bacteria can be part of the initial micro-flora (Razavilar *et al.*, 2012; Huicab-Pech *et al.*, 2017). Additionally, microbial contamination on environmental surfaces can be transferred to the food products directly through surface contact or by vectors, pests or air movement. Bacteria may infect during careless handling of landed fish, its storing and cutting. Among major external sources of bacterial contamination are ice and salt, crushed ice is known to carry heavy bacterial loads and may infect the skin, gills and the gut of live and newly caught fish which can become potential aiders in spoilage

and degradation of quality (Razavilar *et al.*, 2012; Latha and Mohan, 2013; Duarte *et al.*, 2014; Velappan and Munuswamy, 2015; Al-Sheraa, 2018; Barros-Velázquez *et al.*, 2019).

Human infections and diseases that may be caused by pathogens transmitted from fish are quite common and may be influenced by the season, patient's contact with fish and related environment, dietary habits, and the immune system of the individual (Akila and Kumaran, 2018; Huicab-Pech *et al.*, 2017). These pathogens can be divided into two groups: indigenous or autochthonous bacteria which are those that are naturally present on fish such as *Clostridium botulinum* and *Vibrio* spp. (*Vibrio* spp., *V. cholera* and *V. parahaemolyticus*) and those not autochthonous or non-indigenous to the aquatic environment and are due to contamination such as sewage or are introduced to fish during harvesting, processing/handling or storage such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and *Escherichia coli* (Abeyta, 1983; Razavilar *et al.*, 2012; Sahu *et al.*, 2012; Ibemenuga and Okeke, 2014; Pal *et al.*, 2016; Huicab-Pech *et al.*, 2017; Akila and Kumaran, 2018).



**Fig. 2:** Prevalence of Different Types of Pathogenic Bacteria found in fishes

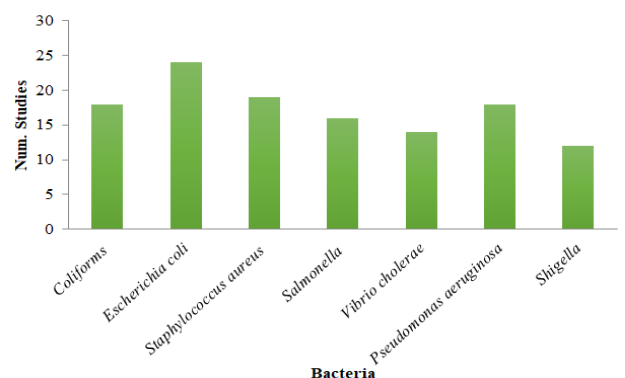
Over 74% of these bacteria that are pathogenic to humans have been found to belong to the Gram negative group (Fig. 1). The autochthonous bacterial flora of fish is mainly dominated by Gram-negative bacteria belonging to genera such as *Acinetobacter*, *Flavobacterium*, *Moraxella*, *Shewanella* and *Pseudomonas* and members of the Enterobacteriaceae family. Members of the families Vibrionaceae (*Vibrio* spp.) and the Aeromonadaceae (*Aeromonas* spp.) are also common aquatic bacteria typical of the fish flora (Strunjak-Perovic *et al.*, 2010; Velappan and Munuswamy, 2015; Huicab-Pech *et al.*, 2017). Gram negative bacteria are among one of the major causes of bacterial disease and are listed as one of the major threats to public health due to the impact and role they play in antibiotic resistance. For example *Aeromonas* spp. causes furunculosis and hemorrhagic septicemia in skin (Huicab-Pech *et al.*, 2017).

Gram-positive microorganisms such as *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Clostridium* spp., and *Lactobacillus* spp. can also be found in 26% of samples as shown in previous studies (Fig. 2). The presence of these bacteria can often be related to the physico-chemical parameters of the aquatic environment as well storage and marketing environments (Huicab-Pech *et al.*, 2017). Gram negative pathogenic bacteria are often predominately associated with food borne infections such as that of fresh fishes and fish products. A study carried out by Huicab-Pech *et al.*, (2017) showed that over 55% of the pathogenic

bacteria isolated from fresh Var. Stirling Tilapia (*Oreochromis Niloticus*) were from Gram negative group. A further study carried out by Jalal *et al.*, in 2017 showed that out of 25 isolated bacteria from fresh and spoiled commercial marine and freshwater fishes 19 were Gram negative with *Vibrio*, spp., *Enterobacter*, spp., *Serratia* spp., and *Aeromonas* spp., being the most dominant genera. These studies correlate with that of Olayemi, *et al.*, (1990) which showed that over 53% of the bacteria isolated from fresh fish were dominated with Gram negative strains. 45% of those isolates belonged to the Enterobacteriaceae family. The remaining 47% positive strains were mainly dominated by *Staphylococcus* and *Micrococcus* genera. Velappan and Munuswamy (2015), in their study recorded similar results whereby 84% of the bacteria present were Gram negative and more than 50% of those belong to the Enterobacteriaceae family. These studies support the fact that majority of the pathogenic bacterial strains belong to the Gram negative family of bacteria (Fig. 2) that are mostly implicated in seafood illness.

#### Major Bacterial Pathogen Found in Fishes and Fish Products

Fish products have been recognized as a major carrier of foodborne pathogens like *Salmonella* spp., *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* as reflected in Table 1 and Fig. 3. Table 1 and 2 identifies common pathogenic bacteria that have been isolated in previous research in sea food products. Disease outbreaks mostly occur due to the ingestion of insufficiently heat-treated fish or products contaminated after or during their processing or preparation. Freezing fish and related products in the seawater, intensive handling and long-time transport can contribute to contamination with microorganisms (Abeyta, 1983). Fig. 3 identifies the bacteria that are among the most prevalent and are normally isolated from fish and fish products.



**Fig. 3:** Prevalence of Major Pathogenic Bacteria Found in Fishes

#### *Vibrio parahaemolyticus* and other vibrios

*Vibrio* occurs naturally in aquatic ecosystems and is part of the natural micro-flora of fish and has been isolated in 14 of the studies used in the assessment of this paper in reference to Fig. 3 (Abeyta, 1983). It is among one of the most pathogenic bacteria found to dominate fishes. The International Association of Microbiology Societies and microbial guidelines as highlighted in Table 3 recommends

that fresh fish should be free of *Vibrio* (0/gm). Additionally, *Vibrio* spp. should not be isolated from fish products since it indicates major gaps in the management practices used during the harvesting and preparation stages to prevent contamination by fishermen and retailers (Sasikala *et al.*, 2019).

*V. parahaemolyticus* has been isolated from sea and estuary waters on all continents with elevated sea water temperatures. *V. parahaemolyticus* is responsible for acute gastroenteritis, however, hospitalization can occur and, on rare occasions, septicaemia may occur. Fish products that have associated with illnesses due to contamination with *V. parahaemolyticus* include sardines (*Sardina pilchardus*), fried mackerel (*Scomber scombrus*) and tuna (*Thunnus thynnus*). These products include both raw or undercooked fish products and cooked products that have been substantially re-contaminated (Abeyta, 1983).

### Enterobacteriaceae

Enterobacteriaceae are Gram-negative facultative anaerobic bacteria that include several human pathogens. These include *Salmonella* spp., *Escherichia coli*, *Shigella* spp., and *Yersinia* spp. These bacteria are widespread in soil, on plant surfaces and in digestive tracts of animals and can occur as result of pollution via sewage and indicate fecal contamination. In Europe Enterobacteriaceae has been widely used for years as indicators of food quality and indices of food safety (Geetha *et al.*, 2014; Jalal *et al.*, 2017; Akila and Kumaran, 2018).

### *Escherichia coli*

*Escherichia coli* is among one of the most dominant pathogenic bacteria that has been isolated in fish products as shown in Fig. 3. *E. coli* is a typical example of enteric bacteria causing gastroenteritis. *E. coli* along with other coliforms and bacteria are used as indicator of hygienic conditions during handling and processing of fish. Such organisms should be absent on freshly-caught fish. The contamination of fish products with pathogenic *E. coli* can occur during handling and production process or as a results of harvesting fishes from polluted waters with poor treatment of sewage and irresponsible disposal (Jalal *et al.*, 2017; Akila and Kumaran, 2018). The microbial criteria in most countries recommend that *E. coli* should not exceed specified limits (<10/100g) as listed in Table 3.

### *Salmonella* spp.

*Salmonella* spp., is a highly pathogenic bacteria and does not occur naturally in marine water and its presence is usually due to improper handling, unhygienic and unsanitary conditions (Akila and Kumaran, 2018; Nur *et al.*, 2020). The contamination of this organism comes from terrestrial sources and fish may serve as a vector. They have also been known to survive in polluted tropical waters once introduced and are considered highly pathogenic and should not be present in food products (Geetha *et al.*, 2014; Sasikala *et al.*, 2019). A study carried out by Nur, *et al.*, (2020) highlighted that all five fish species namely *Brama brama*, *Harpadon neherreus*, *Penaeus monodon*, *Puntius chola* and *Amblyphryngodon microlepi* tested positive for significant loads of *Salmonella* spp. Adverse effects of

ingesting seafood with this pathogenic bacteria could result in nausea, vomiting, abdominal cramps, diarrhea, fever, chills, bloody stool among a few. If left untreated, it could spread be on the intestines and result in health effects that could be deadly (Novotny *et al.*, 2004; Miller, 2019). Typhoid fever caused by *Salmonella typhi*, one of the deadliest strain of this bacteria has been known to affect many low income countries that have been severely affected by poor hygienic practices such as that of the use of contaminated food products and water (Miller, 2019). Thus the microbial guidelines highlight in Table 3 notes that the presence of this bacteria should not be present in 25g of sea food products, any deviation highlights concerns in practices utilized handlers.

### *Shigella* spp.

*Shigella* spp. is a bacteria that is specifically host-adapted to humans and higher primates, and its presence in the environment is usually associated with fecal contamination. *Shigella* spp. isolates have been reported to survive for up to 6 months in water and is the known cause of shigellosis, which is an infection of the gut. The great majority of cases of shigellosis are caused by oral-fecal route and waterborne transmission, especially where hygiene standards are low (Geetha *et al.*, 2014; Sasikala *et al.*, 2019). A research by Shahriar, *et al.*, (2019) and Nur, *et al.*, (2020) on fish species including *Brama brama*, *Lates calcarifer*, *Thunnus albacores* and *Argyrosomus amoyensis* have been found to be carriers of this pathogenic bacteria that may have been introduced due to unhygienic practices. The microbial guides are similar to that of *Salmonella* spp., and should not be present in 25 g of the test samples as recommend in Table 3.

### *Pseudomonas* spp.

*Pseudomonas* spp. are psychrotrophic bacteria that are usually found in iced or refrigerated fish. It is one the major spoilage bacteria commonly found in fish (Jalal *et al.*, 2017) along with *Staphylococcus* spp. (Olayemi *et al.* 1990; Osibona and Ezekiel, 2014) as reflected by Fig. 3. *Pseudomonas* spp. are among one of the most prominent bacteria that have been used in the assessment of quality and spoilage of fish and fish products. The isolation of *Pseudomonas* spp. from fish samples is of high importance because of its considerable role as potential pathogen for human and as an indicator of food quality i.e. spoilage and food deterioration especially in sea foods (Begum *et al.*, 2010). The presence of the Gram negative bacteria is normally due to the poor handling, improper storage system and sanitary condition at various steps in fish processing and selling as well as harvesting since it does not occur naturally as part of the fish microflora and its presence is due to secondary contamination. Four species of *Pseudomonas* (*P. fluorescens*, *P. fragi*, *P. lundensis*, and *P. viridiflava*) are the main food spoilage organisms (Begum *et al.*, 2010). Various studies have highlighted the isolation of these bacteria from fish products and comparatively used it as a spoilage indicator and quality index. This was supported by a study carried out by Begum *et al.*, (2010) who used this bacteria as a quality and spoilage index to make comparisons between local markets and super-shops to highlight method in place to prevent deterioration of

highly perishable sea food resource. Cross contamination of this bacterium infection could cause adverse effects to person with compromised health systems which can affect the skin, lungs, blood and ear among the few locations (Cafasso, 2019).

#### *Staphylococcus* spp.

*Staphylococcus* spp. is also not part of the natural micro-flora in fishes. Its presence indicates the contamination of the fish and its natural environment by human beings and warm-blooded animal (Sasikala *et al.*, 2019). The enterotoxins produced by *Staphylococcus aureus* is of major public health importance. This bacterium has the ability to produce several types of toxins many of which are implicated in food poisoning that cause gastroenteritis after consumption of fish products. It is well-documented that man is the main reservoir of *Staphylococcus aureus* which are found on the skin and in the faeces. The presence of *Staphylococci* in foods is directly associated with employees' sanitary practices (Basti *et al.*, 2006; Mgwede *et al.*, 2018; Nur *et al.*, 2020). This bacteria can be used to access the quality of and management practices employed by retailers. Several species of fishes that are canned and frozen collected from local markets showed high prevalence of *Staphylococcus* spp. (Rokibul, *et al.*, 2013). The presence of this bacterium is directly linked to secondary contamination that is mainly due to poor handling and hygienic practices used by handlers.

#### *Listeria monocytogenes*

*L. monocytogenes* is Gram positive, facultative food borne pathogen of humans and animals, capable of surviving under refrigeration conditions, low pH and in high salt concentration. *L. monocytogenes* is generally distributed in the general environment including fresh, marine and coastal waters and live fishes that are present in these areas. Seafood may become contamination during the handling processes. During the early stages of this infection the initial symptoms generally displayed include chilling, nausea, fever and gastroenteritis. Untreated cases may lead to septicemia, meningitis, encephalitis, abortion and occasionally death (Abeyta, 1983; Novotny *et al.*, 2004; Strunjak-Popovic *et al.*, 2010; Razavilar *et al.*, 2012; Sahu *et al.*, 2012). It should be noted that in the microbial guides outlined by the United States (Table 3) notes that *Listeria monocytogenes* should not present in 25 g of test sample. Due to the fact this bacteria can survive at very low temperatures, caution should be exercised when consuming ready to eat products especially those that are refrigerated.

#### *Clostridium botulinum*

*Clostridium botulinum* is mainly found not only in the soils but they are also associated with sewage, rivers, lakes, sea water, fresh meat and fish. *C. botulinum* type E is among one of the stains and is found in marine and lake sediments and in fish intestine; it does not grow or produce toxin in living fish but it is however carried passively. This bacterium becomes dangerous when processing and cooking practices are insufficient to eliminate botulin spores in raw fish (Abeyta, 1983; Novotny *et al.*, 2004; Strunjak-Popovic *et al.*, 2010; Sahu *et al.*, 2012). The toxin produced by *C.*

*botulinum* is among one of the most toxic substances know and ingestion of even a small quantity could result in severe illness and even death. This neurotoxin is a poisonous chemical that exerts its effects on the central nervous system ultimately destroying, paralyzing and adversely affecting nerves or nerve tissues of the host (USDA, 2013). Hence this bacterial spore should be absent in fish and fish products as recommend and highlighted in Table 3.

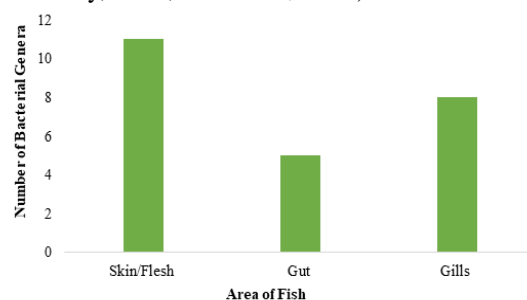
It should be noted that the prevalence of these pathogenic bacteria listed (Table 3) largely depend on the fish species and products from the sources of contamination introduced at different stages of acquiring and processing seafood resources.

#### *Prevalence of Pathogenic Bacteria in Different Parts of Fishes and Fish products*

##### *Micro-flora of fresh fish*

Fresh fish is considered to be sterile, however it still poses some amount of risk since they are carriers of potential bacterial pathogens that may have resulted from the environmental influences (Begum *et al.*, 2010; Adebayo-Tayo *et al.*, 2012). Several parts of a fresh fish may harbor bacterial populations including the skin, gills and digestive tract (Adebayo-Tayo *et al.*, 2012; Razavilar *et al.*, 2012; Giddings *et al.*, 2015; Velappan and Munuswamy, 2015; Akila and Kumaran, 2018; Barros-Velázquez *et al.*, 2019; Sasikala *et al.*, 2019; Nur *et al.*, 2020). However, the bacterial load is not evenly distributed among these areas (Fig. 4). This can be used to access the environment in which these fishes are harvested and sold. The bacterial micro-flora of fresh fish gives an idea of the quality of the fish, the areas that they are harvested from and an overall idea of the condition of the environment and by extension the health of that environment (Jalal *et al.*, 2017; Mgwede *et al.*, 2018; Akter, *et al.*, 2019; Sasikala *et al.*, 2019; Shahriar *et al.*, 2019; Nur *et al.*, 2020).

Fishes may become contaminated when they filter water through their gills, these contaminants can build up in their body over time and can result in health problems after consumption including fever, diarrhea, stomach cramps, nausea and vomiting (Adedeji *et al.*, 2012; Latha and Mohan, 2013). Human activity has a detrimental effect on coastal waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present within the aquatic environment. While handling fishes, it may be contaminated with organisms associated with man such as members of the family Enterobacteriaceae and *Staphylococcus aureus* (Olayemi *et al.*, 1990; Velappan and Munuswamy, 2015; Jalal *et al.*, 2017)



**Fig. 4:** Distribution of Pathogenic Bacteria in Fresh Fishes

The micro-flora of fresh fish includes a wide range of pathogenic bacteria that can arise from many sources that lead to contamination (Table 4). Fresh fish micro-flora especially those of surface organs can be used to assess quality of harvesting environments as well as post-mortem management practices that are utilized by fishermen and retailers (Jalalet *et al.*, 2017; Mgwede *et al.*, 2018; Akter *et al.*, 2019; Sasikala *et al.*, 2019; Shahriar *et al.*, 2019; Nuret *et al.*, 2020). The skin and gills of fresh fish have been known to harbor most of the bacterial loads due to their constant interaction with the external environment as compared to that of the gut (Figure 4). The high load of bacteria on the skin of fishes has been supported by numerous studies. The study by Akila and Kumaran (2018) showed that the pathogenic bacteria were more abundant on the skin of freshly caught *Tilapia zillii* and *Oreochromis mossambicus*, which was  $32.68 \times 10^5$  cfu/g and  $35.12 \times 10^5$  cfu/g respectively. The most common bacterial species that are isolated from fresh fish belong to the Enterobacteriaceae family. The low count of bacteria in the gut was further attributed to the fact that while bacteria can enter into the fish with food and water and accumulate in the intestine of fresh fish, most of them can only remain in this region temporarily due to incompatible environment produced from physical and chemical conditions, and the lethal interactions between bacteria and immune responses of the gut of the host fish.

Further studies by Adebayo-Tayo *et al.*, 2012 showed that most of the bacteria isolated from fresh fish in market conditions yielded higher isolates in the skin (34.8%). This was followed by gills (33.3%) and intestines (31.9%) which had the least bacterial contaminations. They further noted that *E. coli* was the most predominant organism (23.2%). Ibemenuga and Okeke (2014) recorded similar results and found that the bacterial isolates present in 3 different types of fishes were more concentrated in the skins/scales (27.1%) compared to the gut which had 22.7%. *E. coli* was the major species of bacteria isolated in this study followed by member of the *Vibrio* genus. Similar results were also obtained by Velappan and Munuswamy (2015) which showed that the bacterial species were identified for skin mucous had the highest isolation rate of 102 (31.57%) of the total bacteria isolates, while 82 (25.38%) from the intestine, 75 (23.21%) for oral cavity tissues and 44 (13.62%) in the gill. The members of the family Enterobacteriaceae such as *E. coli*, *Salmonella typhimurium* and members of the *Vibrio* family i.e. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* were the common bacterial isolates identified in 50% of the samples.

#### Micro-flora of Spoiled Fishes

Fish undergoing spoilage display many signs such as slime formation, discoloration, changes in texture, off-odors, and production of volatile compounds, which are due to a combination of microbiological, chemical and enzymatic and physical deterioration (Strunjak-Perovic *et al.* 2010; Osibona and Ezekiel, 2014; Velappan and Munuswamy, 2015). The spoilage of fish and fish products are associated with the chemical and biological changes that result after postharvest including techniques related with handling and storage (Jalal *et al.*, 2017).

The microbial load is often a reflection of quality of the processing methods as well as the hygienic practices of the processors and seller being utilized (Abeyta, 1983; Akter and Chowdhury, 2019; Oramadike *et al.*, 2020). One of the major factors contributing to poor quality of the fish and spoilage in retail industry is unhygienic handling, improper storage, physical damage and contact with dirty water and microorganisms (Jalal *et al.*, 2017). Pathogenic bacteria such as *Vibrio* spp., *Shigella* spp., *Salmonella* spp., *Streptococci* spp., *Clostridium* spp., *Pseudomonas* spp. and *Staphylococci* spp. which enter into the fish from their habitat or during fish transportation and storage, have been reported to affect fish quality (Akter and Chowdhury, 2019).

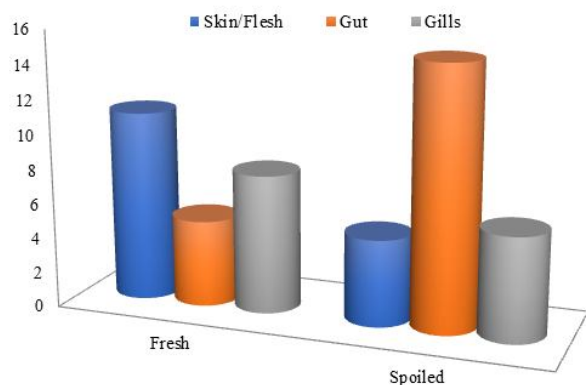
Fish is an ideal substratum for microorganisms due to the availability of appropriate nutrients and moisture that support growth and multiplication of bacteria. Numerous bacteria are present on the surface, on the gills and in the intestines of live fish. Many of them become potential spoilers after the death of fish when the defense system breaks down and the bacteria multiply and invade the flesh (Mgwede, *et al.*, 2018). As the host defense systems start to break down, bacterial invasion becomes easier and hence fresh fish becomes prone to spoilage. This creates an ideal environment for opportunistic bacteria such as lactose degraders, carbohydrates and protease fermenters and positive oxidizers to colonize the different areas of the fish especially in the intestines (Olayemi *et al.* 1990; Rusul and Mahyuddin, 1991; Boulares *et al.*, 2011; Akila and Kumaran, 2018).

The spoiling activities by specific bacteria mainly depend on fish type and chemical composition, feeding habits, the area from where the fish has been harvested and also the types of fishing gears used during harvesting. These factors, along with poor hygienic fish handling practices and improper fish storage conditions, have been known to influence the freshness of fish caught for food purposes (Rokibul *et al.*, 2013). Spoilage may also be accelerated by several factors such as nutrient content, pH, water activity, relative humidity, temperature of the aquatic and marketing environments that support the growth and multiplication of different bacteria that are implicated in spoilage and foodborne illnesses (Mgwede, *et al.*, 2018; Shahriar *et al.* 2019).

Fish contaminated with spoilage bacteria render fish unfit for human consumption. There are specific bacterial pathogens which may be fish borne including *Vibrio parahaemolyticus*, *Vibrio cholerae*, various *Salmonella* and *Shigella* species (Binta *et al.*, 1982; Rokibul *et al.*, 2013; Velappan and Munuswamy, 2015). Unhygienic handling and processing of fish undoubtedly introduces secondary bacterial contaminants such as *E. coli*, *S. aureus* and *Pseudomonas* spp. can also contribute to spoilage (Binta *et al.*, 1982; Rusul and Mahyuddin, 1991; Rokibul *et al.*, 2013; Jalal *et al.*, 2017).

Bacterial degradation increases in the gut region during the spoilage process (Fig. 5) (Begum *et al.*, 2010; Jalal *et al.*, 2017; Akila and Kumaran, 2018). Studies have suggested that intestinal micro-flora are responsible for various food spoilage and contamination of fish flesh and other major parts used for food process (Olayemi *et al.* 1990; Boulares, *et al.*, 2011; Akila and Kumaran, 2018).

In a study carried by Jalal *et al.* (2017), found that the occurrence and distribution of spoilage and pathogenic bacteria varied significantly in different parts of fishes. From the result they collected, 15 bacterial isolates were observed in gut region of spoiled fish; followed by skin (11 isolates) and gill (8 isolates). Spoilage bacteria in the gut and gills are usually present in higher amounts ( $10^3$  and  $10^9$  cfu/g) as compared to that of the skin which records within the ranges of  $10^2$  and  $10^7$  cfu/g (Begum *et al.*, 2010).



**Fig. 5:** Prevalence of Pathogenic Bacteria in Fresh and Spoiled Fishes

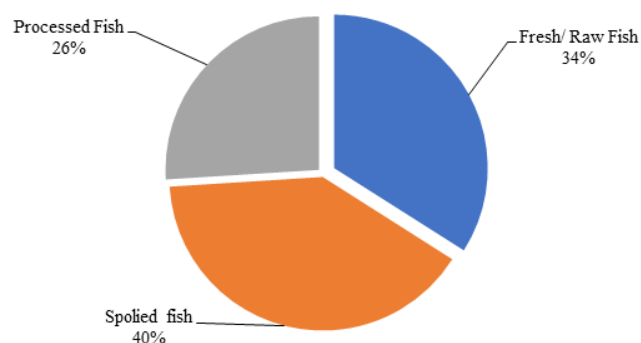
Moreover, the number of spoilage organisms as a proportion of the total bacterial population changes as spoilage proceeds (Jalal *et al.*, 2017). The distribution of these bacteria in different regions of fishes varies as they start to degrade as compared to that of fresh fishes as shown in figure 5. This can be related back to environmental conditions. Fresh fish directly after caught recorded most of its bacterial population in the skin and gills and the least in the gut. However, as spoilage progresses the population increases significantly and reflects more isolates within the gut region of fishes. Sensory analysis revealed that the physical and general quality starts to deteriorate after harvesting. The temperature also increases as fishes start to spoil providing an ideal environment for microbes to multiply (Jalal *et al.*, 2017).

#### *Micro flora of Fresh Fish vs. Spoiled Samples vs. Preserved, Cooked and Frozen*

Management practices utilized by fish handlers from the inception of catching to preparation are vital in order to preserve the integrity of the fishes used for food purposes. Several methods have been implemented over the years in the world for preserving fishes to extend their shelf life including freezing, drying, salting and smoking (Basti *et al.*, 2006). Sun drying of fishes is an effective and well known method of fish preservation. In some countries like Bangladesh, fish drying is among one of the most inexpensive preservation methods. The consumption of improperly cooked fish may sometimes cause fish intoxication that is primarily due to microbes introduced during unhygienic processing, inadequate salting with poor quality salt and poor safety standards in packaging of the fishes (Shahriar *et al.*, 2019).

Freezing and storing at low temperatures slows down bacterial growth and deterioration of fish. The use of good fishing techniques to ensure the fish is not damaged during the capturing process and cooling the fish, with the help of

ice on board, can increase the storage life of fresh fish (Osibona and Ezekiel, 2014).



**Fig. 6:** The Prevalence of Pathogenic Bacteria in Raw, Spoiled and Processed Fish

Frozen fishes are prone to contamination by *Listeria* spp., *S. aureus* and *E. coli* are thermo-stable that contain enterotoxins remain unaffected even after cooking. Due to improper handling recontamination can take place during and after the cooking process leading to food poisoning.

A study by Oramadike *et al.*, (2010) supported these findings highlighting that the total coliforms limits per gram for frozen fish at different supermarkets were within the acceptable range and revealed that once proper post-harvest management techniques are developed and employed contamination and presence of pathogenic bacteria can be reduced significantly.

The comparative study carried out by Shahriar *et al.*, (2019) on the microbial quality of raw, frozen and cooked fishes indicated that the cooked and frozen samples were entirely more satisfactory than the raw samples. This study attempted to determine the effects of cooking on the reduction of existing microorganisms in the fish samples as well as the various consequences that may happen during the frozen condition. The bacterial load was remarkably reduced after cooking, and even the quality was sustained in the frozen state. The major outcome of this study is the finding that cooking at the proper temperature may reduce the microbial spoilage in food and fish.

A further study by Mgwede *et al.*, (2018) on fresh vs parboiled fish also showed reduced presence of bacterial pathogens in samples that were heat treated. Thus, fish products need to be prepared properly to reduce the pathogenic bacterial load that may contaminate during different stages of fish processing. It also pointed out the need for proper management techniques during the handling process so that secondary cross contamination does not occur at any stages of fish processing.

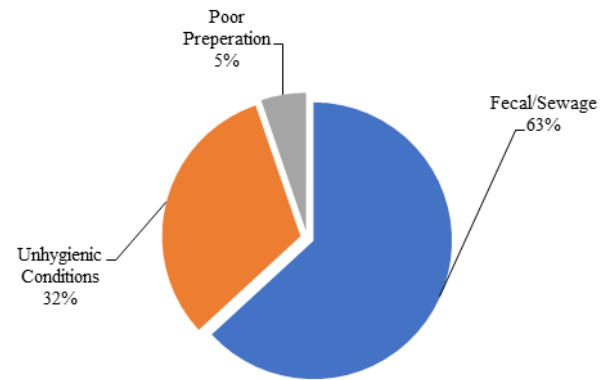
These studies support the data reflected in Fig. 6 which highlights that once fish products are properly prepared under proper hygienic condition the prevalence of pathogenic bacteria is severely decreased.

#### *Major Reasons for Contamination in Fishes*

The quality of fish and fish product used for consumption has been a growing concern over the years. Fresh fish and its related products are very vulnerable to contamination that may infect fishes at different stages of the production from the harvesting to final processing and even in the natural environment (Table 4). Aquatic environments are susceptible to contamination, and in some

areas the water movement is very slow, allowing bacteria to be easily transferred to fish from the water, sediments, and from its feeding behavior and food sources (Latha and Mohan, 2013; Duarte *et al.*, 2014; Al-Sheraa, 2018). These bacteria can infect different parts of the fishes including the skin, gills and digestive tract (Austin, 2002). These contaminants can build up in their body over time and can result in health problems including fever, diarrhea, stomach cramps, nausea and vomiting (Adedeji *et al.*, 2012; Latha and Mohan, 2013).

Contaminated environments often cause infection in these animals which can later be transferred to humans during the handling process and consumption. The associated microorganisms that are transferred to fish may sometimes cause disease in the person that handles or consumes same (Noornissabegum and Revathi, 2014). The post-harvest losses may be high due to several reasons, including poor handling of the catch such as rough handling, harvesting of fish from contaminated waters, unhygienic handling, time lag between fish catch, icing and proper preservation, lack of or delayed icing, poor quality water used in markets by fish handlers, poor hygiene of the market place, all contribute to accelerated spoilage as reflected in Table 4 (Sahu *et al.*, 2014). It is important to identify the effect of different post-harvest treatments on the quality of fish to evaluate how effective they are on the preservation of fresh fish and shelf life (Osibona and Ezekiel, 2014; Huicab-Pech *et al.*, 2017). Bacterial foodborne illnesses can be attributed to factors such as fishing from polluted waters, improper refrigeration facilities, improper practice of strict sanitation procedures in fishing vessels, processing plants and storage facilities, diseased food handlers, and improper cooking time (Abeyta, 1983).



**Fig. 7:** The Major Reasons Related to Source of Contaminations

From previous research analyzed, it was noted that most of the contaminations were due to the market environment and sewage (Fig. 7). High number of fecal indicators from the family of Enterobacteriaceae especially *E. coli* (Table 4) were present in significant numbers. This highlights that many of these market environments had very poor sanitary conditions, poor management practices and improper handling of sewage treatment and disposal. The identification of significant loads of coliform (Fig. 3) also indicate possible presence of other highly pathogenic bacteria such as *Salmonella* spp., and *Shigella* spp. It should also be pointed out that *Staphylococcus* spp., was among the second highest bacterial load that was isolated from most of these studies (Fig. 3), which point directly to the management techniques that are used during the handling, preparation and marketing of fish and its products (Binta *et al.*, 1982; Abeyta 1983; Razavilar *et al.*, 2012; Latha and Mohan, 2013; Pal *et al.*, 2016).

**Table 1:** Studies on Pathogenic Bacteria in Fish and Fish Products

Author	Year	Pathogenic Bacteria	Area Sampled
Abeyta	1983	<i>Staphylococcus aureus</i> , <i>E. coli</i> , <i>Enterococci</i> , <i>Vibrio parahaemolyticus</i> , <i>Yersinia enterocolitica</i>	Sea food markets
Rusul <i>et al.</i>	1991	<i>Coliforms</i> , <i>Vibrio parahaemolyticus</i>	Market
Basti <i>et al.</i>	2006	<i>Coliforms</i> , <i>Vibrio parahaemolyticus</i> , <i>Staphylococcus</i> spp.	Sea farms
Oramadike <i>et al.</i>	2010	<i>Escherichia coli</i> , <i>Salmonella</i> , <i>Shigella</i> , <i>Vibrio</i> spp.	Supermarket
Begum <i>et al.</i>	2010	<i>Pseudomonas</i> spp., <i>Vibrio</i> spp., <i>Salmonella</i> spp., <i>coliforms</i> , <i>Escherichia coli</i>	Market
Strunjak-Perovic <i>et al.</i>	2010	<i>Salmonella</i> spp., <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Vibrio cholera</i> , <i>V. parahaemolyticus</i>	Sea and market
Boulares <i>et al.</i>	2011	<i>Lactobacillus</i> spp., <i>Pseudomonas</i> spp.	Ponds
Nilla <i>et al.</i>	2012	<i>Vibrio</i> spp., <i>E. coli</i> , and <i>Staphylococcus</i> spp.	Market
Adebayo-Tayo <i>et al.</i>	2012	<i>Bacillus</i> spp., <i>Shigella</i> spp., <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>Pseudomonas</i> spp., <i>Enterococcus</i> spp., <i>Salmonella</i> spp.	Market
Sahu <i>et al.</i>	2012	<i>E. coli</i> , <i>S. aureus</i> , <i>V. cholera</i> , <i>V. parahaemolyticus</i> , <i>Salmonella</i>	Market
Razavilar <i>et al.</i>	2012	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella</i> spp., <i>Vibrio</i> spp., <i>Listeria monocytogenes</i> .	Ponds
Rokibul <i>et al.</i>	2013	<i>Pseudomonas</i> spp., <i>Salmonella</i> spp., <i>Shigella</i> spp., <i>Vibrio</i> spp., <i>Listeria</i> spp., <i>Staphylococcus</i> spp., <i>Clostridium</i> spp.	Market
Latha <i>et al.</i>	2013	<i>Pseudomonas</i> , <i>Aeromonas</i> , <i>Aeromonas</i> , <i>Enterobacteriaceae</i> , <i>Micrococcus</i> , <i>Bacillus</i> , and <i>Lactobacillus</i>	Markets
Reddy <i>et al.</i>	2014	<i>Vibrio</i> spp., <i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>S. aureus</i>	Landing site
Geetha <i>et al.</i>	2014	faecal coliform, <i>Pseudomonas</i> spp., <i>Salmonella</i> spp., <i>Vibrio</i> spp., <i>Shigella</i> spp., <i>Staphylococcus</i> spp.	Landing site
Giddings <i>et al.</i>	2015	<i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>S. aureus</i>	Market
Velappan <i>et al.</i>	2015	<i>Escherichia coli</i> , <i>Salmonella typhimurium</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Vibrio</i> spp.	Landing site



Huicab-Pech <i>et al.</i>	2017	<i>Aeromonas</i> spp., <i>Pseudomonas</i> spp., <i>Edwardsiella</i> spp., <i>Flexibacter</i> spp., <i>Flavobacterium</i> spp., <i>Arthrobacter</i> spp., <i>Enterococcus</i> spp., <i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>Streptococcus</i> spp., <i>Vibrios</i> spp.	Production units
Jalal <i>et al.</i>	2017	<i>Vibrio</i> , <i>Enterobacter</i> , <i>Serratia</i> , and <i>Aeromonas</i> , <i>Staphylococcus</i> spp.	Market
Mgwede <i>et al.</i>	2018	<i>Enterobacter</i> , <i>Klebsiella</i> , <i>Pseudomonas</i> , <i>E. coli</i> , <i>Enterococcus</i> , <i>Shigella</i> , <i>Salmonella</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermis</i> , <i>Vibrio cholerae</i> , <i>Vibrio parahaemolyticus</i> .	Markets
Al-Sheraa, Akila, et al.	2018	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> spp.	Fishing boats
	2018	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Salmonella typhi</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>Shigella dysenteriae</i>	Lake
Shahriar <i>et al.</i>	2019	<i>E. coli</i> , <i>Klebsiella</i> , <i>Staphylococcus</i> spp., <i>Shigella</i> spp., <i>Salmonella</i> spp., <i>Pseudomonas</i> spp. <i>Vibrio</i> spp.	Market
Akter <i>et al.</i>	2019	<i>Salmonella</i> spp., <i>Shigella</i> spp. and <i>Vibrio cholerae</i> <i>Escherichia coli</i> coliforms	Capture and landing site
Sasikala <i>et al.</i>	2019	<i>Salmonella</i> spp., <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp., <i>Vibrio</i> spp., coliforms	Landing site
Nur <i>et al.</i>	2020	<i>E. coli</i> , <i>Shigella</i> spp., <i>Klebsiella</i> spp., <i>Vibrio</i> spp., <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp.	Market

**Table 2:** Major pathogenic bacteria isolated from different fish species

Author	Year	Fish species	Type of fish/ Fish product	Dominant Pathogenic bacteria Isolated	Reason for contamination
Abeyta	1983	Shellfish, finfish	fresh	<i>Escherichia coli</i>	Sewage contamination
Rusul <i>et al.</i>	1991	<i>Rastrelliger</i> spp. , <i>Selaroides ieptolepsis</i> , <i>Nemipterns</i> spp., <i>Scomberomorus</i> spp., <i>Megalaspis cordyla</i> , <i>Decapterns</i> spp., <i>Parastromatens niger</i> , <i>Selar crumenophthalmus</i>	Fresh	Coliforms	Poor handling/ sewage contamination
Basti <i>et al.</i>	2006	<i>Alosa kessleri</i> , <i>Hypophthalmichthys molitrix</i> . <i>Liza aurata</i>	Fresh, smoked, salted Frozen	<i>Listeria monocytogenes</i>	poor preparation
Oramadike <i>et al.</i>	2010	Barracudas, Croakers, Sole, Salmon, Mullet and Red mullet	Fresh and Frozen	<i>Escherichia coli</i>	Samples were of accepted levels
Begum <i>et al.</i>	2010	<i>Barbodes sarana</i> , <i>Labeo rohita</i> , <i>Oreochromis niloticus</i> , <i>Sperata seenghala</i> , <i>Corica soborna</i>	Fresh and Frozen	<i>Pseudomonas</i> spp.	poor handling and improper storage system
Strunjak-Perovic <i>et al.</i>	2010	<i>Dicentrarchus labrax</i> , <i>Sparus aurata</i> , <i>Sprattus sprattus</i> , <i>Sardina pilchardus</i> , <i>Scorpaena scrofa</i> , <i>Thunnus thynnus</i> , <i>Merluccius merluccius</i> , <i>Mullus surmuletus</i>	Fresh and frozen	Enterobacteriaceae	poor handling and improper storage system
Boulares <i>et al.</i>	2011	<i>Chelon labrosus</i> , <i>Merlangius Merlangus</i> , <i>Solea solea</i> , <i>Sardina pilchardus</i> , <i>Scomber scombus</i> , <i>Mullus surmuletus</i> , <i>Sparus pagrus</i>	Fresh	Fecal coliforms	Sewage contamination
Nilla <i>et al.</i>	2012	<i>Amblypharyngodon mola</i>	Fresh and frozen	<i>Escherichia coli</i>	Sewage contamination
Adebayo-Tayo et al.	2012	<i>Arius hendelotic</i>	Fresh	<i>Escherichia coli</i>	Sewage contamination
Sahu <i>et al.</i>	2012	Rohu, Hilsa, Pomfret, Horse Mackerel	Fresh	<i>Escherichia coli</i>	unhygienic handling practice/sewage
Razavilar <i>et al.</i>	2012	<i>Hypophthalmichthys molitrix</i>	Fresh	<i>Escherichia coli</i>	Sewage contamination
Rokibul <i>et al.</i>	2013	Tuna and salmon	Frozen and canned fish	<i>Staphylococcus</i> spp.	Poor Hygienic conditions

Latha <i>et al.</i>	2013	<i>Glossogobius giuris</i> , <i>Labeo rohita</i>	Fresh	<i>Pseudomonas</i> spp.	Poor handling practices
Reddy <i>et al.</i>	2014	<i>Upeneus vittatus</i> , <i>Nemipterus japonicus</i> , <i>Priacanthus hamrur</i>	Fresh	Fecal coliforms	Sewage contamination
Geetha <i>et al.</i>	2014	<i>Upeneus vittatus</i> , <i>Nemipterus japonicus</i> , <i>Priacanthus hamrur</i>	Fresh	<i>Vibrio cholera</i>	Contamination via Marine environment
Giddings <i>et al.</i>	2015	<i>Hoplosternum littorale</i> , <i>Cichlasoma bimaculatum</i> , <i>Hoplias malabaricus</i>	Fresh	<i>Staphylococcus</i> spp.	Poor Hygienic conditions
Velappan <i>et al.</i>	2015	<i>Lutjanus campechanus</i> , <i>Sardinella longiceps</i>	Fresh	<i>V. parahaemolyticus</i>	Poor Hygiene quality
Huicab-Pech <i>et al.</i>	2017	<i>Oreochromis Niloticus</i>	Fresh	<i>Bacilli</i>	Poor aquaculture management
Jalal <i>et al.</i>	2017	<i>Lutjanus sanguineus</i> , <i>Lates calcarifer</i> , <i>Pangasius pangasius</i>	Fresh	<i>Staphylococcus</i> spp.	Poor market conditions
Mgwede <i>et al.</i>	2018	<i>Engraulicypris sardella</i>	Fresh and Parboiled	-	Poor Hygiene quality
Al-Sheraa,	2018	<i>Pampus argenteus</i> , <i>Brachirus orientalis</i> , <i>Acanthopagrus latus</i>	Fresh	<i>Escherichia coli</i>	Sewage contamination
Akila <i>et al.</i>	2018	<i>Tilapia zillii</i> , <i>Oreochromis mossambicus</i>	Fresh	<i>P. aeruginosa</i>	Poor hygienic quality
Shahriar <i>et al.</i>	2019	<i>Pampus chinensis</i> , <i>Lates calcarifer</i> , <i>Thunnus albacores</i> , <i>Argyrosomus amoyensis</i>	Fresh	<i>Escherichia coli</i>	Sewage and poor post-harvest contamination
Akter <i>et al.</i>	2019	<i>Ompok pabda</i>	Fresh	<i>Escherichia coli</i>	unhygienic conditions
Sasikala <i>et al.</i>	2019	<i>Trichiurus lepturus</i> , <i>Upeneus vittatus</i> , <i>Leiognathus equulus</i>	Fresh	<i>Escherichia coli</i>	Sewage contamination
Nur <i>et al.</i>	2020	<i>Brama brama</i> , <i>Harpadon neherreus</i> , <i>Penaeus monodon</i> , <i>Puntius chola</i> , <i>Amblyphryngodon microlepi</i>	Raw and sun-dried	<i>Escherichia coli</i>	Sewage contamination

**Table 3:** Microbial Criteria for Bacterial Pathogens Found in Raw and Cooked Fish (Raymond and Ramachandran, 2019)

Countries	Microbiological criteria/guidelines/specification/maximum limits	
	Raw fish (fresh and Frozen)	Cooked/prepared
US	<i>E. coli</i> : MPN of 230/100 g <i>Salmonella</i> : ND in 25 g <i>Listeria monocytogenes</i> : ND in 25 g <i>Vibrio cholerae</i> : ND in 25 g <i>Vibrio parahaemolyticus</i> : 1x 10 <sup>4</sup> /g <i>Vibrio vulnificus</i> : Absence <i>S. aureus</i> : <105—106/g <i>Clostridium botulinum</i> : Absence of viable spore, vegetative cells, toxin	<i>E. coli</i> ETEC: 1x 10 <sup>3</sup> /g <i>Salmonella</i> : ND in 25 g <i>Listeria monocytogenes</i> : ND in 25 g <i>Vibrio cholerae</i> : ND in 25 g <i>Vibrio parahaemolyticus</i> : <1x 10 <sup>4</sup> /g <i>Vibrio vulnificus</i> : Absence <i>S. aureus</i> : <105—106/g <i>Clostridium botulinum</i> : Absence of viable spore, vegetative cells, toxin
INDIA	<i>E. Coli</i> : <20 g-1 <i>S. aureus</i> : <100 g-1 <i>Salmonella</i> : ND in 25 g <i>Shigella</i> : ND in 25 g <i>Vibrio cholerae</i> : ND in 25 g <i>Vibrio parahaemolyticus</i> : ND in 25 g	<i>E. Coli</i> : ND in 25 gm <i>S. aureus</i> : ND in 25 gm <i>Salmonella</i> : ND in 25 g <i>Shigella</i> : ND in 25 g <i>Vibrio cholerae</i> : ND in 25 g <i>Vibrio parahaemolyticus</i> : ND in 25 g
EU	<i>E. Coli</i> : <230/100 g <i>S. aureus</i> : <103/g	<i>Salmonella</i> : ND in 25 <i>V. parahaemolyticus</i> : ND in 25 g <i>L. monocytogenes</i> : ND in 25 g <i>S. aureus</i> : <20 g-1
South Africa	<i>E. coli</i> Type 1: <10/100g <i>Salmonella</i> : < 20/g <i>Shigella</i> : < 20/g <i>Vibrio cholerae</i> : < 20/g <i>Vibrio parahaemolyticus</i> : < 20/g Coagulase-positive <i>S. aureus</i> : < 20/g	<i>E. coli</i> Type 1:< 20/g <i>Salmonella</i> : <20/g <i>Shigella</i> : < 20/g <i>Vibrio cholerae</i> : < 20/g <i>Vibrio parahaemolyticus</i> : < 20/g Coagulase-positive <i>S. aureus</i> : < 20/g

**Table 4:** Major Reasons contributing to contamination in fishes

Sample area	Major pathogenic bacteria	Reason for contamination	Number of samples Tested	Estimated Positive %	References
Sea food markets	<i>Escherichia coli</i>	Sewage contamination	287	60.45	Abeyta 1983
Market	Coliforms	Poor handling/ sewage contamination	40	-	Rusul <i>et al.</i> 1991
Sea farms	<i>Listeria monocytogenes</i>	poor preparation	67	10	Basti <i>et al.</i> 2006
Supermarket	<i>Escherichia coli</i>	Samples were of accepted levels	-	-	Oramadike <i>et al.</i> 2010
Market	<i>Pseudomonas</i> spp.	poor handling and improper storage system	-	-	Begum <i>et al.</i> 2010
Sea and market	Enterobacteriaceae	poor handling and improper storage system	240	66.6	Strunjak-Perovic <i>et al.</i> 2010
Ponds	Fecal coliforms	Sewage contamination	80	-	Boulares <i>et al.</i> 2011
Market	<i>Escherichia coli</i>	Sewage contamination	24	62.5	Nilla <i>et al.</i> 2012
Market	<i>Escherichia coli</i>	Sewage contamination	-	71	Adebayo- <i>et al.</i> 2012
Market	<i>Escherichia coli</i>	unhygienic handling practice/sewage	100	17	Sahu <i>et al.</i> 2012
Ponds	<i>Escherichia coli</i>	Sewage contamination	-	78.57	Razavilar <i>et al.</i> 2012
Market	<i>Staphylococcus</i> spp	Poor Hygienic conditions	20	-	Rokibul <i>et al.</i> 2013
Markets	<i>Pseudomonas</i> spp.	Poor handling practices	-	90	Latha <i>et al.</i> 2013
Landing site	Fecal coliforms	Sewage contamination	-	-	Reddy <i>et al.</i> 2014
Landing site	<i>Vibrio cholera</i>	Contamination via Marine environment	-	35.4	Geetha <i>et al.</i> 2014
Market	<i>Staphylococcus</i> spp	Poor Hygienic conditions	18	-	Giddings <i>et al.</i> 2015
Landing site	<i>V. parahaemolyticus</i>	Poor Hygiene quality	50	99.6	Velappan <i>et al.</i> 2015
Production units	<i>Bacilli</i>	Poor aquaculture management	-	55	Huicab <i>et al.</i> 2017
Market	<i>Staphylococcus</i> spp	Poor market conditions	58	-	Jalal <i>et al.</i> 2017
Markets	-	Poor Hygiene quality	40	-	Mgwede <i>et al.</i> 2018
Fishing boats	<i>Escherichia coli</i>	Sewage contamination	80	50	Al-Sheraa, 2018
Lake	<i>P. aeruginosa</i>	Poor hygienic quality	-	-	Akila <i>et al.</i> 2018
Market	<i>Escherichia coli</i>	Sewage and poor post-harvest contamination	12	-	Shahriar <i>et al.</i> 2019
Capture and landing site	<i>Escherichia coli</i>	unhygienic conditions	-	-	Akter <i>et al.</i> 2019
Landing site	<i>Escherichia coli</i>	Sewage contamination	-	-	Sasikala <i>et al.</i> 2019
Market	<i>Escherichia coli</i>	Sewage contamination	50	-	Nur <i>et al.</i> 2020

## CONCLUSION

The presence of potentially pathogenic organisms in fishes and their products is of concern to public health. Contamination may affect not only the health of fish stocks, but also raise public health concerns as fish and fish products can be a potential source of human pathogenic bacteria and the cause of diseases. Fish is often contaminated with foodborne pathogens such as *Escherichia coli*, *Pseudomonas* spp., *Streptococcus* spp., *Vibrio* spp., *C. botulinum*, *C. perfringens*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp. *Listeria monocytogenes* and *Vibrio* spp. which reflects the micro-flora of the surrounding water and marketing environment. Numerous factors including human activities and improper sewage disposal can contaminate water sources. This along with poor hygiene during capture, handling, transportation and processing of fish could introduce these pathogenic bacteria into fishes. The hazard of these microorganisms is increased with the specific abilities of these bacteria to survive and progress in the environment. Thus, it is recommended that a thorough surveillance of the microbiological status of fish

and their products be done for the safety of consumers. Constant inspections and long term monitoring and evaluation of local markets by authorities could pave a way in minimizing the spread of bacterial pathogenicity in human and aid in the development of management plans that help in preventing disease out breaks and preserve consumer's health.

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## APPENDIX

### References

- Fig. 1 (Oramadike *et al.*, 2010; Mgwede *et al.*, 2018; Shahriar *et al.*, 2019; Nur *et al.*, 2020)
- Fig. 2 Abeyta, 1983, Rusul *et al.*, 1991, Basti *et al.*, 2006, Oramadike *et al.*, 2010, Begum *et al.*, 2010, Strunjak-Perovic *et al.*, 2010, Boulares *et al.*, 2011, Nilla *et al.*, 2012, Adebayo-Tayo *et al.*, 2012, Sahu *et al.*, 2012, Razavilar *et al.*, 2012, Rokibul *et al.*, 2013, Latha *et al.*, 2013, Reddy *et al.*, 2014, Geetha *et al.*, 2014, Giddings *et al.*, 2015, Velappan *et al.*, 2015, Huicab-Pech *et al.*, 2017, Jalal *et al.*, 2017, Mgwede *et al.*, 2018, Al-Sheraa, 2018, Akila, *et al.*, 2018, Shahriar *et al.*, 2019, Akter *et al.*, 2019, Sasikala *et al.*, 2019, Nur *et al.*, 2020
- Fig. 3 Abeyta, 1983, Rusul *et al.*, 1991, Basti *et al.*, 2006, Oramadike *et al.*, 2010, Begum *et al.*, 2010, Strunjak-Perovic *et al.*, 2010, Boulares *et al.*, 2011, Nilla *et al.*, 2012, Adebayo-Tayo *et al.*, 2012, Sahu *et al.*, 2012, Razavilar *et al.*, 2012, Rokibul *et al.*, 2013, Latha *et al.*, 2013, Reddy *et al.*, 2014, Geetha *et al.*, 2014, Giddings *et al.*, 2015, Velappan *et al.*, 2015, Huicab-Pech *et al.*, 2017, Jalal *et al.*, 2017, Mgwede *et al.*, 2018, Al-Sheraa, 2018, Akila, *et al.*, 2018, Shahriar *et al.*, 2019, Akter *et al.*, 2019, Sasikala *et al.*, 2019, Nur *et al.*, 2020
- Fig. 4 Jalal *et al.*, 2017
- Fig. 5 Begum *et al.*, 2010, Jalal *et al.*, 2017, Akila and Kumaran, 2018
- Fig. 6 Oramadike *et al.*, 2010, Shahriar *et al.*, 2019, Mgwede *et al.*, 2018
- Fig. 7 Abeyta, 1983, Rusul *et al.*, 1991, Basti *et al.*, 2006, Oramadike *et al.*, 2010, Begum *et al.*, 2010, Strunjak-Perovic *et al.*, 2010, Boulares *et al.*, 2011, Nilla *et al.*, 2012, Adebayo-Tayo *et al.*, 2012, Sahu *et al.*, 2012, Razavilar *et al.*, 2012, Rokibul *et al.*, 2013, Latha *et al.*, 2013, Reddy *et al.*, 2014, Geetha *et al.*, 2014, Giddings *et al.*, 2015, Velappan *et al.*, 2015, Huicab-Pech *et al.*, 2017, Jalal *et al.*, 2017, Mgwede *et al.*, 2018, Al-Sheraa, 2018, Akila, *et al.*, 2018, Shahriar *et al.*, 2019, Akter *et al.*, 2019, Sasikala *et al.*, 2019, Nur *et al.*, 2020