

Microbiological contamination of imported frozen fish marketed in Eastern Province of Saudi Arabia

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Abstract

The present study was conducted to determine the bacterial contamination and prevalence of foodborne pathogens in imported frozen fish. A total of 450 imported fish samples [65 catfish (*Pangasius pangasius*), 45 mirgal (*Cirrhinus mrigala*), 135 tilapia (*Oreochromis niloticus*), 50 carfoo (*Cyprinus carpio*), 75 Rohu (*Labeo rohita*), 35 milk fish (*Chanos chanos*) and 45 Mackerel (*Rastrelliger brachysoma*)] were randomly purchased from different supermarkets. The samples originated from 5 countries, namely, Thailand (n=260), India (n=75), Vietnam (n=35), Myanmar (n=35) and Bahrain (n=45). The isolation and identification were done according to standard bacteriological analytical methods. Out of total samples, 49.1% (95%CI: 44.52 to 53.72) were positive for foodborne pathogens. The highest percentage of positive bacterial contamination were from Vietnam milk fish, 65.7% (95%CI: 49.15 to 79.17), followed by Catfish from Thailand, 58.5% (95%CI: 46.34 to 69.64) and Mirgal from Thailand, 57.8% (95%CI: 43.30 to 71.03). The leading bacterial contaminations and foodborne pathogens in these samples were: *E. coli* (18.6%), *Enterococci* (14.4%), *Pseudomonas* (14%) and *Salmonella* (16.8%), respectively. Correspondingly, the country with the highest odds for pathogens is Thailand, 1.0472 (95% CI: 0.8217 to 1.3347) and the fish with the highest odds for pathogens is Tilapia 0.6667 (95%CI: 0.4000 to 1.1112) followed by catfish, 1.4074 (95% CI: 0.8636 to 2.2938). In Saudi Arabia and to date, comprehensive data regarding the bacterial contamination and prevalence of foodborne pathogens in imported frozen freshwater fish are limited or not available.

Keywords

Bacterial contamination
Pathogenic bacteria
Imported fish
Food safety
Saudi Arabia

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Introduction

Aquaculture is the fastest growing source of protein production and 52.5 Million of tones accounted for 45.6% of fish production in 2008 (FAO, 2011). There is a global trade in aquaculture products has considerably increased in recent decades and the expansion of aquaculture production, particularly from Asia, has the potential to meet most of the growing global demand for fish and fishery products (FAO, 1997), and half of the global seafood consumption will originate from aquaculture by 2020 (FAO, 1995). According to Food and Agriculture Organization (FAO), aquaculture supplies about 50% of the global demand for fish and fishery products with about 90% of the aquaculture products coming from the Asian region (FAO, 2011).

Aquaculture, owing to its rapid expansion in an actively mobile environment and extensive movement of animals between different sites, creates

conditions in which pathogens can spread (Murray, 2013). Human pathogens, mainly bacterial and parasites may be associated with fish and shellfish products. Bacterial pathogens of fecal origin can be transmitted to aqua-cultured products in the pond or during subsequent handling and processing as a result of inadequate hygienic conditions (Noor Uddin *et al.*, 2013).

Fishery products have been recognized as a major carrier of food-borne pathogens (Yucel and Balci, 2010; FAO, 2011). Pathogenic bacteria associated with fish and fishery product can be categorized into three general groups: Indigenous bacteria that belong to the natural micro-flora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*); Enteric bacteria (Non-Indigenous bacteria) that are present due to fecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*); and bacterial contamination during processing,

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storage or preparation for consumption (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp.) (Lyhs, 2009). Improper storage and handling of fishery products may also lead to increased growth of spoilage bacteria such as *Lactobacillus* spp., *Proteus* spp., *Shewanella putrefaciens*, and *Pseudomonas* spp. (Sorum, 2006; Thomas and Matthews, 2008; Al Bulushi et al., 2010).

Detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety, since the food safety is becoming a global health concern and the foodborne diseases take a major crisis on health (Zhao et al., 2001). Accordingly, the present study was undertaken to examine the total microbial contamination and the presence of foodborne pathogens in imported frozen fish marketed in Eastern Province of Saudi Arabia.

Materials and Methods

Fish sample collections and preparation

Between December 2012 and February, 2013, a total of 450 imported frozen fish samples [65 catfish (*Pangasius pangasius*), 45 mirgal (*Cirrhinus mrigala*), 135 tilapia (*Oreochromis niloticus*), 50 carfoo (*Cyprinus carpio*), 75 Rohu (*Labeo rohita*), 35 milk fish (*Chanos chanos*) and 45 Mackerel (*Rastrelliger brachysoma*)] originated from 5 countries, namely, Thailand (n=260), India (n=75), Vietnam (n=35), Myanmar (n=35) and Bahrain (n=45) were randomly purchased from different supermarkets in Eastern Province of Saudi Arabia and examined for bacterial contamination and significant foodborne pathogens.

The majority of countries had only one type of fish, with the exception of Thailand (catfish, mirgal, tilapia, carfoo and rohu). All the collected fish samples were found with labeled information indicating the country of origin, labeled as frozen belly gutted or non-gutted fish, date of production, date of expiry, weight, storage temperature (-18°C). Samples were purchased from each supermarket and transported on ice to the Microbiology Research Laboratory in University of Dammam. Upon arrival, the samples were kept intact on ice and analyzed within 3 to 5 hours of collection and were examined in aseptic conditions. 25 g of each sample (fish gills, intestines parts and skin) were placed into a stomacher bag containing 225 ml broth recommended for isolation of each foodborne pathogens according to the methods described in the U.S. Food and Drug Administration Bacteriological Analytical Manual (US-FDA, 2003) with slight modification. All the sample portions

of fish were homogenized for 2 minutes using a stomacher (Seward Stomacher 400 Circulator, UK).

Bacterial isolation and identification

The isolation of *E. coli* were carried out according to the published protocol with modifications (Zhao et al., 2001) Briefly, 25 g of each samples were placed into a stomacher bag containing 225 ml of EC broth (Oxoid, UK) and homogenized using a stomacher (Seward Stomacher 400 Circulator, UK) for 2 min and incubated for 18–24 h at 35°C. After enrichment incubation, 0.1 ml was streaked onto CHROMagar O157:H7, MacConkey agar and Brilliance *E. coli* Agar (Oxoid, UK). Three to five isolated pink to red colonies and purple colonies from each sample on MacConkey agar and Brilliance *E. coli* Agar were sub-cultured on Trypticase soy agar (TSA) to carry out biochemical tests. The positive indole and oxidase-negative isolates were further confirmed by using API 20E (bioMérieux, France).

Salmonella was isolated following a previous method (Lestari et al., 2009; Huang et al., 2012) with some modifications. Briefly, 25 g of samples were placed into a stomacher bag containing 225 ml of buffered peptone water and homogenized using a stomacher (Seward Stomacher 400 Circulator, UK) for 2 min and incubated for 18–24 h at 37°C. From this nonselective pre-enrichment, 0.1 ml and 1 ml were, respectively, transferred into 10 ml of Rappaport-Vassiliadis broth incubated for 24 hours at 42°C and 10 ml of selenite cystine broth incubated at 37°C. A drop from each selective enrichment broth was streaked onto selective Hektoen agar, Rambach agar and CHROMagar *Salmonella* agar (CHROMagar, Paris, France) plates and incubated for 24–48 hours at 37°C. Suspected colonies on selective agar plates were purified and bio-typed by using biochemical tests and API 20E strips (BioMérieux, Marcy, France). *Salmonella enterica* subsp. *enterica* Serovar *enteritidis* from American Type Culture Collection (ATCC) 13076 was used as a reference strain for CHROMagar, biochemical tests and API 20E strips. Up to two to three confirmed *Salmonella* isolates per samples were stored in TSB with 20% glycerol at -80°C.

Vibrio species was isolated following a previous method (Elhadi et al., 2004). Briefly, each 25 g of fish sample taken in 225 ml of alkaline peptone water (APW; Oxoid) and homogenized using a stomacher (Seward Stomacher 400 Circulator, UK) for 2 min and the homogenized samples were incubated at 37°C for 6 hours and for 18–24 hours to isolate *Vibrio cholerae* and other *Vibrio* species, respectively. The enrichment culture after incubation period was then

streaked onto thiosulfate–citrate–bile salt–sucrose (TCBS) agar (Oxoid) and incubated at 37°C for 24 h. Presumptive *Vibrio* colonies were then streaked onto CHROMagar *Vibrio* and incubated for 24 hours at 37°C. The presumptive *Vibrio* colonies were confirmed with the API 20E test and salt tolerance test at 0, 3, 8, 10, and 12% NaCl at 37°C for 24 hours.

Listeria CHROMagar medium were used to isolate *Listeria* from frozen imported fish samples with the modified protocol of ISO 11290 (Scotter et al., 2001). Briefly, 25 g of fish sample was mixed in stomacher machine with 225 ml of Fraser broth (Oxoid, UK) and incubated at 35°C for 24 hours. Full-loop from the incubated Fraser broth was streaked onto *Listeria* CHROMagar (Biomerieux, France) plates and incubated at 35°C for 48 hours. The suspected isolates to be *Listeria* were subjected to standard biochemical tests.

Staphylococcus aureus were isolated following a previous method (Hammad et al., 2012). A 25 g fish sample was added to 225 ml volume of double-strength enrichment broth (trypticase soy broth supplemented with 10% NaCl and 1% sodium pyruvate) and mixed using stomacher machine. After 24 h incubation at 37°C, enrichment broth was streaked onto CHROMagar *S. aureus*, Baird-Parker (Oxoid, UK) agar and mannitol salt agar (Oxoid, UK) containing cefoxitin (4 µg/ml; Oxoid, UK) and plates were incubated for 24 h at 37°C. After incubation, three to five presumptive *Staphylococcus* colonies from each plate were transferred to trypticase soy agar plates. Presumptive *Staphylococcus* was identified to species level based on colony morphology, hemolysis, and gram staining and slide coagulation tests.

Enterococci were isolated according to a previous method (Brtkova et al., 2010). Briefly, 25 g of fish samples were mixed with 225 ml of Buffered Peptone Water (Oxoid, UK) and incubated at 37°C for 24 h. After incubation, one loop full of the overnight suspension was inoculated on bile Esculin azide agar (Oxoid) and incubated at 37°C for 24 h. Colonies grown on agar with a dark brown halo and morphologically resembling enterococci were isolated. Suspected colonies of *Enterococcus* spp were transferred to a non-selective medium containing sheep blood (Columbia agar, Oxoid). Presumptive isolates were Gram-positive and haemolytic negative cocci and did not produce catalase and oxidase enzymes. The suspected enterococci isolates were identified by using a serial number of biochemical tests as published in previous study (Knudtson and Hartman, 1992).

The isolation of *Bacillus cereus* from frozen imported fish samples was carried out by using

Bacillus cereus selective agar (Oxoid, UK) with the polymyxin B as a supplement. Briefly, 25 g of fish sample was mixed in stomacher machine with 225 ml of tryptic soy broth (Oxoid, UK) and incubated at 35°C for 24 h. Full-loop from the incubated broth was streaked onto *Bacillus cereus* selective agar plates and incubated at 35°C for 24 h. The suspected isolates to be *Bacillus cereus* were subjected to gram stain and standard biochemical tests (US-FDA, 2012).

Other enteric bacteria (*Enterobacter*, *Citrobacter*, *Klebsiella* and *Proteus*) and *Pseudomonas* were isolated using MacConkey Agar, Rambach Agar and CHROMagar and whereas cetrinide agar were used for *Pseudomonas*. Briefly, 25 g of samples (fish gills, intestines parts and skin) were placed into a stomacher bag containing 225 ml of buffered peptone water broth (Oxoid, UK) and homogenized using a stomacher (Seward Stomacher 400 Circulator, UK) for 2 min and incubated for 18–24 h at 35°C. After enrichment incubation, 0.1 ml was streaked onto MacConkey Agar, Rambach Agar to isolate enteric bacteria, and 0.1 ml was streaked onto CHROMagar and cetrinide agar to isolate *Pseudomonas*. The suspected colonies were subjected for serial number of biochemical tests and confirmed by using API 20 E kits (bioMe'rieux, France) for enteric bacteria and API20 NE kits were used for *Pseudomonas*.

Results

Out of all 450 fish samples included in the study, 49.1% (95%CI: 44.52 to 53.72) were positive for bacterial contamination and foodborne pathogens. Among these, the highest percentage of positive samples was milk fish from Vietnam, 65.7% (95%CI: 49.15 to 79.17), followed by Catfish from Thailand, 58.5% (95%CI: 46.34 to 69.64) and Mirgal from Thailand, 57.8% (95%CI: 43.30 to 71.03) (Table 1). Mackerel fish from Bahrain and Tilapia from Thailand showed the least percentage of positive samples (Table 1).

The total numbers of bacterial contamination and foodborne pathogens isolates recovered from imported frozen fish in this study were 1643 (Table 2 and 3). The bacterial contamination includes *E. coli*, *Enterococci*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Shewanella putrefaciens* (Table 2), and the foodborne pathogens includes *Salmonella*, *Staphylococcus aureus*, *V. alginolyticus* and *Bacillus cereus* (Table 3). The leading bacterial contamination were: *E. coli* (18.6%), *Enterococci* (14.4%) and *Pseudomonas* (14%) (Fig 1), and foodborne pathogens were *Salmonella* (16.6%) and *Bacillus cereus* (9.4 %) (Fig 2), respectively.

Table 1. Prevalence of bacterial contamination in imported freshwater fish

Country of origin	Fish type	No. of samples	No. of positive samples	Prevalence (%)	95% CI
Thailand	Catfish	65	38	58.5	46.34 to 69.64
	Mirgal	45	26	57.8	43.30 to 71.03
	Tilapia	60	24	40	28.57 to 52.63
	Carfoo	50	28	56	42.31 to 68.84
	Rohu	40	17	42.5	28.51 to 57.80
India	Tilapia	75	32	42.7	32.10 to 53.95
Vietnam	Milk Fish	35	23	65.7	49.15 to 79.17
Myanmar	Rohu	35	15	42.9	27.98 to 59.14
Bahrain	Mackerel	45	18	40	27.02 to 54.55
Total		450	221	49.1	44.52 to 53.72

Table 2. Distribution of bacterial contaminations isolated from fish according to imported country of origin

Bacterial contaminants	Country of origin									
	Thailand					India	Vietnam	Myanmar	Bahrain	Total No. (%) of isolates (n=1678)
	Catfish	Mirgal	Tilapia	Carfoo	Rohu	Tilapia	Milk Fish	Rohu	Mackerel	
<i>E. coli</i>	65	36	50	15	33	39	28	24	22	312 (18.6)
<i>Enterococci</i>	57	13	32	21	9	28	35	19	27	241 (14.4)
<i>S. putrefaciens</i>	0	0	0	0	0	0	0	0	17	17 (1)
<i>Pseudomonas</i>	40	7	70	11	5	24	40	6	32	235 (14)
<i>Proteus</i>	9	2	13	6	8	5	12	0	8	63(3.8)
<i>Klebsiella</i>	17	5	28	9	0	6	13	0	7	85 (5)
<i>Enterobacter</i>	12	9	30	12	0	14	18	0	10	105 (6.3)
<i>Citrobacter</i>	25	17	22	11	15	8	11	0	0	109 (6.5)
Total No. of isolates	225	89	245	85	70	124	157	49	123	1167

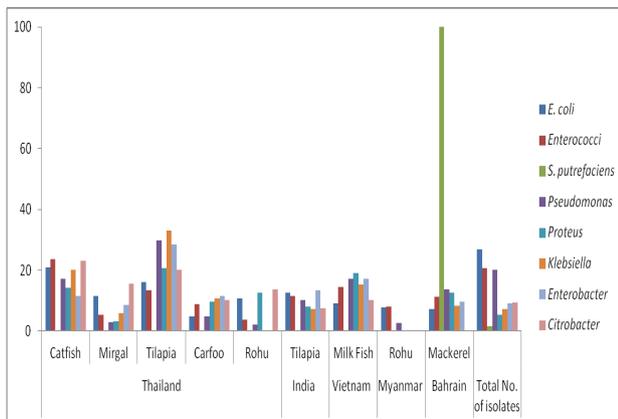


Figure 1. Distribution of bacterial contaminations isolated from fish according to imported country of origin

All the fish samples were examined in this study were negative for *Listeria*, *V. vulnificus* and *V. parahaemolyticus*. (Table 3).

In total, the bacterial contamination and foodborne pathogens account for over 65% of all the frozen fish samples. Tilapia and Catfish from Thailand had the highest numbers of foodborne pathogens (Table 3). Correspondingly, the country with the highest number of bacterial contamination is Thailand (Fig 1 and Table 2) and the fish with the highest foodborne pathogens is Tilapia, followed by catfish, (Fig 2 and Table 3). The present study found the imported frozen fish samples were not contaminated with *Listeria* and pathogenic *Vibrio* species.

Discussion

This is the first study to carry out microbiological investigation of bacterial contamination and foodborne pathogens in raw freshwater fish samples imported to Saudi Arabia. The vast majority of fish samples analyzed in this study were frozen freshwater fish such as catfish, tilapia, mirgal, carfoo, rohu and milk fish, and another sample type was aquatic mackerel fish which originated from Middle East and represent 10% of the overall total of fish samples. In this study, there was no efforts made to samples certain or specific countries, though the 90% of samples originated from the seafood exporters suppliers in South East Asian Countries. In this, *Vibrio* species such as *V. cholerae*, *V. parahaemolyticus* and *Vibrio vulnificus* did not recover from the fish samples investigated in this study, since these organisms present only in marine fish and this study mainly concentrated on imported frozen freshwater fish.

Investigation of foodborne pathogens in food is playing a significant role in prevention of foodborne pathogens transmission (WHO, 2008). The microbial quality of fish is an important aspect of food safety. Raw fish products have been reported as vehicles for foodborne illness (Uradznski *et al.*, 2007). Fishery products are very important for human nutrition and health benefits worldwide, and can also act as a source of foodborne pathogens (Kromhout *et al.*, 1985;

Table 3: Distribution of foodborne pathogens isolated from fish according to imported country of origin

Pathogens	Country of origin									
	Thailand					India	Vietnam	Myanmar	Bahrain	Total No. (%) of isolates (n=1678)
	Catfish	Mirgal	Tilapia	Carfoo	Rohu	Tilapia	Milk Fish	Rohu	Mackerel	
<i>Salmonella</i>	48	25	58	24	18	45	35	12	17	282 (16.8)
<i>V. parahaemolyticus</i>	0	0	0	0	0	0	0	0	0	0
<i>V. vulnificus</i>	0	0	0	0	0	0	0	0	0	0
<i>V. alginolyticus</i>	0	0	0	0	0	0	0	0	18	18 (1.1)
<i>Listeria</i>	0	0	0	0	0	0	0	0	0	0
<i>S. aureus</i>	18	3	12	0	0	9	11	0	0	53 (3.2)
<i>B. cereus</i>	42	30	45	0	0	28	13	0	0	158 (9.4)
Total No. of isolates	108	58	115	24	18	82	59	12	35	511

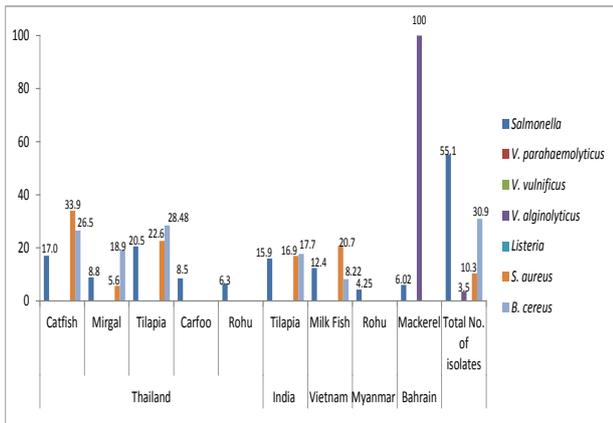


Figure 2. Distribution of foodborne pathogens isolated from fish according to imported country of origin

Darlington and Stone, 2001). The majority of reported seafood-associated out breaks are caused by toxins (biotoxins and histamine) and viruses (noroviruses and hepatitis A virus) but fish and shellfish may also be a vehicle for pathogenic bacteria, referred to as indigenous, or derived from polluted waters and/or from post capture contamination (WHO, 2000; FAO, 2014).

In this study, the highest numbers of *Salmonella* isolates were isolated from tilapia, catfish (imported from Thailand), tilapia (imported from India) and milkfish imported from Vietnam, where, the lowest number of *Salmonella* isolates were isolated from rohu fish (imported from Myanmar), mackerel fish (imported from Bahrain), rohu and carfu fish (imported from Thailand). *Salmonella* contamination in fish and fishery products has also been reported from other countries like India, Mexico, Thailand, Hong Kong, Spain and Turkey (Herrera *et al.*, 2006; Kumar *et al.*, 2009; Pamuk *et al.*, 2011; FAO, 2014.). The highest *Salmonella* incidence in fishery products was determined in Central Pacific and African countries while it was lower in Europe and including Russia, and North America (New and Csavas, 1995). In the Asia-Pacific region, cultured fishes are fed by both commercial and homemade feeds (fresh feed

material or farm feed material). According to FAO, homemade feeds are used to reduce cost of production (FAO, 2010). Homemade feed is usually made from chicken viscera, kitchen refuse, chicken bone, and other food waste materials (New and Csavas, 1995). Such feeds can be a source of pathogenic bacteria such as *Salmonella* spp. (Lunestad *et al.*, 2007) which can be transmitted to freshwater fish and ultimately to consumers. In a recent study in Malaysia, (Budiati *et al.*, 2013) reported that feeds such as chicken offals and spoiled eggs can posed a potential source of *Salmonella* spp. and the high risks associated with the dissemination of antibiotic resistance genes among bacteria associated with catfish, tilapia and environment of aquaculture systems. Salmonellosis is one of the most important diseases in both humans and animals and has been described as the second most common cause of foodborne bacterial human disease worldwide (Gast, 2008). It is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* spp. occur annually worldwide leading to 155,000 deaths each year (Majowicz *et al.*, 2010). A significant increase in the number of *Salmonella* infections has been observed in many countries over the past decade (Yang *et al.*, 2010). Globally, the most prevalent serovars in humans are *Salmonella enterica* serovars Typhimurium and Enteritidis (Hendriksen *et al.*, 2011).

The overall *E. coli* contamination in fish samples examined in the present study yielded a total of 312 isolates, and the highest numbers of isolates were recovered from catfish, tilapia and mirgal fish (imported from Thailand), tilapia (imported from India) and milkfish (imported from Vietnam) (Table 2). *E. coli* has been found in fishery and other seafood at prevalence rates varying widely by country to country. *E. coli* prevalence and isolation rates were reported more higher in India and Vietnam, likely due to poor raw food hygiene (Thampuran *et al.*, 2005; Van *et al.*, 2008). In other hand, several studies (Samadpour *et al.*, 1994; Teophilo *et al.*,

2002) have reported the detection of either Shiga toxin-producing *E. coli* or enterotoxigenic *E. coli* in seafood, and found them to be absent in some studies (Thampuran *et al.*, 2005; Van *et al.*, 2008; Pao *et al.*, 2008).

All fish samples examined in this study were found negative for the presence of *Vibrio* species and *Listeria*, except *Vibrio alginolyticus* was recovered from mackerel (marine fish) imported from Bahrain. In this study *Listeria* was examined, since there are several reports indicate that fish and fishery products can be frequently contaminated with *L. monocytogenes* (FAO, 1999). The presence of *Listeria* in fish has been reported by some researchers, and the incidence rates vary widely (Yucel and Balci, 2010). There are variations in the incidence of *Listeria* in seafood and fishery products in different parts of the world and can be attributed to secondary contaminations during handling, storage, and transportation (Teophilo *et al.*, 2002). There are several reports concerning the etiological role of *V. cholerae*, *V. vulnificus*, and *V. parahaemolyticus* in foodborne diseases (Jaksic *et al.*, 2002). In this study majority of fish samples examined were freshwater fish. However, *Vibrio* species are halophilic bacteria and could present in marine fish (Baffone *et al.*, 2005). Available information suggests that *L. monocytogenes* occurs naturally in freshwater fish and seafood from polluted waters but is unlikely to occur on fish specimens from open seawater (Huss *et al.*, 2000). In the present study, all *Listeria* species were found absent in all fish samples examined, and the obtained data were in agreement with one study in Turkey reported that some *Listeria* species were not found in samples of freshwater fish (Yucel and Balci, 2010). Some studies elsewhere reported the incidence of *L. monocytogenes* in fish and fishery products from temperate parts of the world is approximately 50% (Dillon and Patel, 1992, Fuchs and Reilly, 1992). The present study did not find any incidence of the major *Vibrio* species such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in freshwater fish samples, similar to study reported elsewhere (Yucel and Balci, 2010).

Staphylococcal species occur as commensal colonizers of the skin and mucous membranes of different species of animals and humans; *staphylococci* are not part of the normal fish microflora (Huss, 1988). The presence of *Staphylococci* on fish is an indication of post-harvest contamination due to poor personnel hygiene or disease in fish (Austin and Austin, 2007). In the present study, the highest figure of *S. aureus* were from catfish, tilapia (imported from Thailand), milk fish (imported

from Vietnam), and tilapia (imported from India). The presence of the *S. aureus* in examined frozen freshwater fish in this study is an indication of the possibility of contamination during the processing or packing the product and the contamination could be the result of a combination of improper handling, packaging, and cross contamination (Herrera *et al.*, 2006; Vázquez-Sánchez *et al.*, 2012). *S. aureus* is one of the major bacterial agents causing foodborne diseases in humans worldwide (Le-Loir *et al.*, 2003). *Staphylococcal* food poisoning is usually self-limiting and resolves within 24 to 48 h after onset. Most cases are therefore not reported to healthcare services. As a result, the actual incidence of staphylococcal food poisoning is known to be much higher than reported (Lawryniewicz-Paciorek *et al.*, 2007). *Staphylococcal* food poisonings result from the ingestion of food containing staphylococcal enterotoxins (SEs) preformed enterotoxigenic strains (Kérouanton *et al.*, 2007). SEs are resistant to proteolysis and heat-stable, so the presence of SEs involves a significant food safety risk (Omoe *et al.*, 2005).

In the present study, there were several bacterial contaminants detected in examined frozen fish samples, such as, *Enterococcus*, *Bacillus cereus*, *Pseudomonas*, *Shewanella putrefaciens*, *Klebsiella*, *Enterobacter (Cronobacter)*, *Proteus* and *Citrobacter* (Table 1). The highest figures were with *Bacillus cereus* were isolated from tilapia and catfish imported from Thailand and tilapia imported from India (Table 2). *B. cereus* is an important foodborne pathogen and has been recognized as a causative agent of food poisoning. *B. cereus* has been linked to foodborne emetic and diarrheal syndromes (Schoeni and Wong, 2005; Das *et al.*, 2009; Fernández-No *et al.*, 2011) and also fatal meningitis (Evreux *et al.*, 2007). This organism is also responsible for spoilage of different food products (Meer *et al.*, 1991). Since *B. cereus* is a spore forming organism, there is a risk of its transmission through heat-treated and processed food products. Some isolates of *B. cereus* can grow at refrigerated temperature (Te Giffel *et al.*, 1997; Valero *et al.*, 2007), and spore can survive at high temperature. All fish samples examined in this study were positive for *Pseudomonas* and *Shewanella putrefaciens* (Table 2). *Pseudomonas* and *Shewanella putrefaciens* are known as the major spoilers of ice stored tropical freshwater fish and marine fish stored at psychotropic temperature (Gram and Dalgaard, 2002; Rasmussen *et al.*, 2002). Further studies from these findings are under process to carry out antimicrobial susceptibility testing and molecular characterization of the *E. coli*, *Salmonella*, *Staphylococcus aureus* and *Enterococci* isolated

from imported frozen freshwater fish.

Conclusion

To date none of the studies have been performed to detect the presence of foodborne pathogens in imported frozen fish in Saudi Arabia. This study is a comprehensive survey on the presence of bacterial contaminants and some of the major foodborne pathogens in imported frozen freshwater fish imported to Eastern Province of Saudi Arabia. These results findings highlight potential food safety hazards associated with imported frozen freshwater fish and warrant further large scale studies to continuously monitor the microbiological quality and safety of imported fish and other seafood products. Study results indicate that imported frozen freshwater fish harbors some of the enteric pathogens and consumption of uncooked freshwater fish should be avoided, and proper cooking of fish is encouraged to avoid foodborne illness.

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