



The Prevalence of *Campylobacter*, *Listeria* and *Salmonella* Species in Freshwater and Salt-Water Fish in Eastern Turkey

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ARTICLE INFO

Article history:

Received 6 April 2017

Accepted 14 May 2017

Available online 1 June 2017

Keywords:

Campylobacter,
Fish,
Listeria,
PCR,
Salmonella,

ABSTRACT

A total of 154 samples of marine (n=51) and freshwater fish (n=103) were obtained from fish markets in Elazig Province of eastern Turkey. These samples were tested for *Campylobacter*, *Listeria* and *Salmonella* using culturing and biochemical methods. *Campylobacter* failed to be detected in any freshwater or marine fish samples. *Listeria* was detected in 22 and 14 of gill and skin samples from freshwater fish, respectively. *L. innocua* was isolated at a higher prevalence (14.6%) than *L. ivanovii* (5.8%) and *L. monocytogenes* (1%) from the gill samples of freshwater fish. In skin samples, *L. innocua* was detected at higher prevalence (9.7%) than *L. ivanovii* (2.9%) and *L. welshimeri* (1%). However, two (1.9%) of the intestine samples of freshwater fish were found to be positive for *L. innocua*. In addition, *L. monocytogenes* isolate yielded a positive band by PCR. *Listeria murrayi* was the most commonly isolated species with a prevalence of 9.8% and 5.9% from the skin and gill samples of marine fish, respectively. However, the lowest prevalence (3.9%) of *L. innocua* was found from skin samples of marine fish only, but none of the intestine samples of marine fish were tested positive for *Listeria* spp. *L. monocytogenes* was not isolated in any marine fish samples. The results indicate that fish can carry a pathogenic *Listeria* species. However, *Campylobacter* and *Salmonella* were not detected in marine fish samples suggests that fish pose no or little risk to the human population in Elazig Province in eastern Turkey.

1. Introduction

Listeria and *Salmonella* are two of the most important bacterial food-borne pathogens worldwide and human infection has considerable economic and public health costs (Momtaz, 2013). *Listeria* and *Salmonella* spp. have been indicated to be present in a wide range of seafood products including shrimp (Rocourt,

2000, Wan Norhana, 2010), crab (Rahimi, 2012; Rahimi, 2013), cold-smoked rainbow trout (Pagadala, 2012), fish products (Ericsson, 1997; Elhadi, 2014) and lobster (Rahimi, 2013; Yildirim, 2004). The prevalence of *Listeria monocytogenes* (*L. monocytogenes*) in raw fish has been shown to be low, varying from 0-1%

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(Autio, 1999; Johansson, 1999) to 10% (Jemmi, 1994). In Europe, the prevalence of *L. monocytogenes* in fish was 3% (Davies, 2001). In contrast, Miettinen (2005) demonstrated that the prevalence of *L. monocytogenes* and *Listeria* spp. isolated from unprocessed fresh rainbow trout was found to be 14.6% and 35%, respectively (Miettinen, 2005).

Campylobacteriosis related to the consumption of fish or fish products is relatively rare (Novotny, 2004). In case-controlled studies, the consumption of fish has not been determined as a risk factor (Novotny, 2004) and the incidence of *Campylobacter* spp. in fish products has been shown to be low (2.3%) (LoewenherzLuning, 1996).

Listeria and *Salmonella* carriage in fish is potentially of high importance for public health; however, there is currently little data on the prevalence of *Listeria* and *Salmonella* spp. in fish in Turkey (Ertas, 2005; Yucel, 2010; Mus, 2014; Onmaz, 2015; Ikiz, 2016). In addition, there is no known study on the identification of *Campylobacter* spp. from fish samples in Turkey. The goal of the current study was to detect the prevalence of *Campylobacter*, *Listeria* and *Salmonella* species in raw fish in the Elazig Province of eastern Turkey.

2. Materials and Methods

2.1. Samples

A total of 154 fish samples (103 freshwater and 51 marine) were purchased from different fish markets in Elazig Province of eastern Turkey. Fish samples were individually bagged, placed on ice and immediately transported to the Microbiology Laboratory at Firat University, Faculty of Veterinary Medicine, and processed within 2 hours. The outer surface of fish was washed three times with sterile water, then disinfected by a 70 % ethyl alcohol wipe. The skin, gills and intestine were removed aseptically using a sterile scalpel blade (Traoré, 2014). The fish skin, gill and intestine samples were weighed separately, and these were placed separately in a sterile stomacher bag. A 100-ml volume of sterile 0.1% (wt/vol) peptone water (Oxoid, Basingstoke, UK) was added to each sample and homogenized using a stomacher (Interscience, 78860 St Nom-France) at high speed (Yücel, 2010).

2.2. Culture of *Campylobacter*, *Listeria* and *Salmonella* species

For *Campylobacter* isolation, one gram of skin, gill and intestine samples from each fish was added to 9 ml of Bolton Broth with Bolton Supplement (CM0983 with SR0183 and SR0048 (Oxoid, Basingstoke, UK) and 5% lysed horse blood (SR0048C, Oxoid, UK) and incubated at 42°C for 48 h in microaerophilic conditions using the Campy-Gen gas generating kit (Oxoid, UK). The enrichment broth was streaked onto modified Charcoal Cefoperazone Deoxycholate agar (mCCDA) (Oxoid CM 739, SR 155) and incubated at 42°C for 24-48 h in microaerophilic conditions (Chai, 2007). The plates were assessed for the presence of presumptive *Campylobacter* colonies. Isolates were kept for further identification using biochemical tests; catalase, oxidase, growth at 25°C and 43°C, H₂S production in triple sugar iron agar, susceptibility to nalidixic acid and cephalotin, hippurate hydrolysis and hydrolysis identification of indoxyl acetate (Wainø, 2003; Chai, 2007; Tan, 2008).

Listeria spp. were isolated from whole skin, gill and intestine samples from each fish according to ISO 11290 protocol (Scotter, 2001). Identification of the presumptive colonies was performed using biochemical tests; Gram staining, catalase test, acid production from mannitol, glucose, xylose, rhamnose, α -methyl-D-mamoside, motility test at 25°C and 37°C, Methyl Red/Voges-Proskauer test, nitrate reduction, beta-hemolytic activity, hydrolysis of esculin, and CAMP test with *Staphylococcus aureus* (Aygun, 2006; Momtaz, 2013). *Listeria monocytogenes* isolate was kept for further confirmation using PCR. The isolates were banked at -80°C in 1 mL of Brain Heart Infusion broth (Oxoid, Basingstoke, UK) with 15% (v/v) buffered glycerol for subsequent analysis.

The isolation of *Salmonella* spp. from skin, gill and intestine samples of each fish was carried out as described in Bacteriological Analytical Manual (Andrews, 2011). The suspect colonies were confirmed by biochemical tests: oxidase, catalase, carbohydrate utilization from lactose, dulcitol, sucrose and salicin Simon's citrate, indole test, urease, Methyl Red-Voges Proskauer, motility test on Sulfide Indol Motility (SIM) medium (Merck), H₂S

4. Discussion

Data on the prevalence of *Listeria* and *Salmonella* in fish in eastern Turkey is limited. However, to our knowledge, there is no known study on the identification of *Campylobacter* spp. from fish in Turkey. In addition, these microorganisms are generally not identified in Turkey's routine diagnostic laboratories (Yücel, 2010).

Adesiyun (1993) reported that 28 (5.8%) of 480 fish samples were found to be positive for *Listeria* spp. with fish showing the highest prevalence (14.8%). In addition, the same author showed that 14 (2.9%) and 9 (1.9%) of the samples were detected to be positive for *L. innocua* and *L. monocytogenes*, respectively (Adesiyun, 1993). The proportion of *L. monocytogenes* from marine and freshwater fish in earlier studies ranged from 0% within the United States and Greece to 77% in the United Kingdom (Davies, 2001; Papadopoulos, 2010; Püllela, 1998). The prevalence of *L. monocytogenes* at 1% in this current study is lower than the earlier reported values in France (65%), the United Kingdom (77%), Portugal (25%), Finland (14.6%), Denmark (8.6%), the United States (7.8%) and Latvia (13%) (Davies, 2001; Miettinen, 2005; Norton, 2001; Vogel, 2001; Terentjeva, 2015) but it is in agreement with another study in Turkey which also reported a low prevalence (2%) (Ikiz, 2016). Another study conducted in Elazığ Province of eastern Turkey showed a prevalence of 6.6% (Ertaş, 2005).

A study carried out in Nagpur, Central India showed that *L. monocytogenes*, *L. seeligeri*, *L. grayi* and *L. welshimeri* in 200 freshwater fish samples were found in 26 (67%), 8 (21%), 3 (8%) and 2 (5%), respectively, and the authors reported that *L. monocytogenes* in this samples was the most predominant species (Jallewar, 2007). Rahimi (2012) demonstrated that *Listeria* species were detected in 11.7%, 7.5%, 4.2%, and 6.6% of fresh shrimp, fresh fish, frozen fish and frozen shrimp samples, respectively and that *L. innocua* and *L. monocytogenes* were identified in 5.7% and 1.9% of the fresh and frozen seafood samples, respectively (Rahimi, 2012). In a study performed by Das (2013), *Listeria* spp. was found in 32.3%, 27.1%, and 5% of fresh, frozen, and dry fish samples, respectively and *L. innocua* was determined at a higher prevalence

(41.7%) than *L. monocytogenes* (1.2%) and other *Listeria* spp. in the tropical seafood and environmental samples (Das, 2013). The current study indicated that *L. innocua* (26.2%) in freshwater fish was the most prevalent among *Listeria* spp. and this is in accordance with previous studies (Adzitey, 2010; Das, 2013; Ammar, 2014). This might suggest an importance impact since the competition between species may be at the basis of the low incidence of other *Listeria* species. The isolation of *L. innocua* in fishery products is considered as a good indicator for *L. monocytogenes* (Ammar, 2014).

A study conducted in Turkey showed that *Listeria* spp. was present in 30% of freshwater fish and 10.4% of marine fish (Yücel, 2010). The most common species identified in freshwater fish was *L. monocytogenes* making up 44.5% of the species isolated (Yücel, 2010). In marine fish, *L. murrayi* was found at a higher percentage and made up 83.5% of *Listeria* spp. isolated (Yücel, 2010). The great variability in prevalence of *Listeria* spp. in seafood was probably related to the types of marine organism collected (fish, shrimp, crab, oyster and lobster), sample type (whole organism, intestines, skin, flesh or gills), number of samples, techniques of sampling, culturing and identification (Miettinen, 2005; Momtaz, 2013). Geographic region, weather conditions and seasonal variations will also influence the results (Miettinen, 2005; Momtaz, 2013). The contamination of fish with *Listeria* spp. in this study may be due to the lack of hygiene standards during capture and processing, and polluted waters.

In the current study, the isolation rate of *Listeria* spp. from different parts of the fish was examined. It has been reported that gills are an excellent niche for bacteria despite this area being part of the immunological system (Yücel, 2010). Here, *Listeria* spp. was at the highest prevalence on gill samples (22/103, 21.4%) of freshwater fish yet failed to be isolated from the intestines of marine fish. Miettinen (2005) found that 43 gills of 510 fish were positive for *L. monocytogenes*, while only 1 skin and 1 visceral sample were positive for *L. monocytogenes*. However, some researchers indicated that they could not recover *L. monocytogenes* from any of freshwater fish samples (Karunasagar, 1992; Kamat, 1994; Papadopoulos, 2010). Contrary to

our results, a study carried out in Turkey reported that the highest incidence of *Listeria* spp. was found in skin samples of freshwater fish and the gill samples of marine fish (Yücel, 2010). Consistent with data derived from one publication (Yücel, 2010) in Turkey, *L. murrayi* (90.5%) was the most common species obtained from marine fish but in contrast to an earlier report that the most common species being *L. monocytogenes* (38%) in skin samples of freshwater fish.

No *Salmonella* was detected from either freshwater fish or marine fish in this study. Our findings are consistent with the previous reports focusing on countries such as France, Czech Republic, Portugal, Great Britain, the United States, and Latvia (Andreji, 2006; Davies, 2001; Hudecova, 2010; Püllela, 1998; Terentjeva, 2015). In contrast, the isolation of *Salmonella* in fish was detected in several countries such as India (14.25%), Iran (10.4%), Kenya (31.7%), Nigeria (11.5%), Turkey (12.5%) and Egypt (3.9%) (David, 2009; Hatha, 1997; Raufu, 2014; Shabarinath, 2007; Youssef, 1992; Ikiz, 2016). In a study performed to determine the prevalence of *Salmonella* spp. from frozen freshwater fish imported from five different countries such as Thailand, India, Bahrain, Myanmar and Vietnam, 140 of 223 samples examined were found to be positive for *Salmonella* spp (Elhadi, 2014). The same authors demonstrated that 140 isolates of *Salmonella* spp. were obtained from at least seven various types of frozen freshwater fish (Elhadi, 2014). A research performed in Elazig Province of eastern Turkey reported that *Salmonella* spp. was isolated from 3.45% of raw fish samples (Ertaş, 1999).

In the present study, *Campylobacter* spp. was not detected in either freshwater or marine fish samples. Similarly, in Finland, neither *C. jejuni* nor *C. coli* were isolated in any samples of vacuum-packaged, cold-smoked, or hot-smoked fish products (Lyhs, 1998). This data is consistent with other studies in Vietnam and Iran which failed to isolate *C. jejuni* from fish samples (Ha, 2006; Raeisi, 2017). However, tuna salad has been cited as the source of *C. jejuni* (O:33) infections (Roels, 1998), but this may be due to cross-contamination during preparation, rather than being present in the raw fish (Novonty, 2004). Seafood may not be a natural reservoir of *Campylobacter* (Tan, 2008),

however, previous studies have indicated a prevalence of 3.4% in shrimps (Adesiyun, 1993), and 2.3% in shellfish (Whyte, 2004).

Conclusion

This study has demonstrated a low prevalence (1%) of *L. monocytogenes* in freshwater fish but none contained *Campylobacter* spp. and *Salmonella* spp. This may be due to the fact that fish in Turkey usually are not consumed undercooked. Appropriate hygiene standards during capture and processing and obtaining seafood from clean waters are required to further reduce the risk of human infection along with thorough cooking of fish products.

Acknowledgements

This study was presented as a poster in XI. National Congress of Veterinary Microbiology (With International Guest Speakers, October 21-24, 2014, Kemer, Antalya).

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